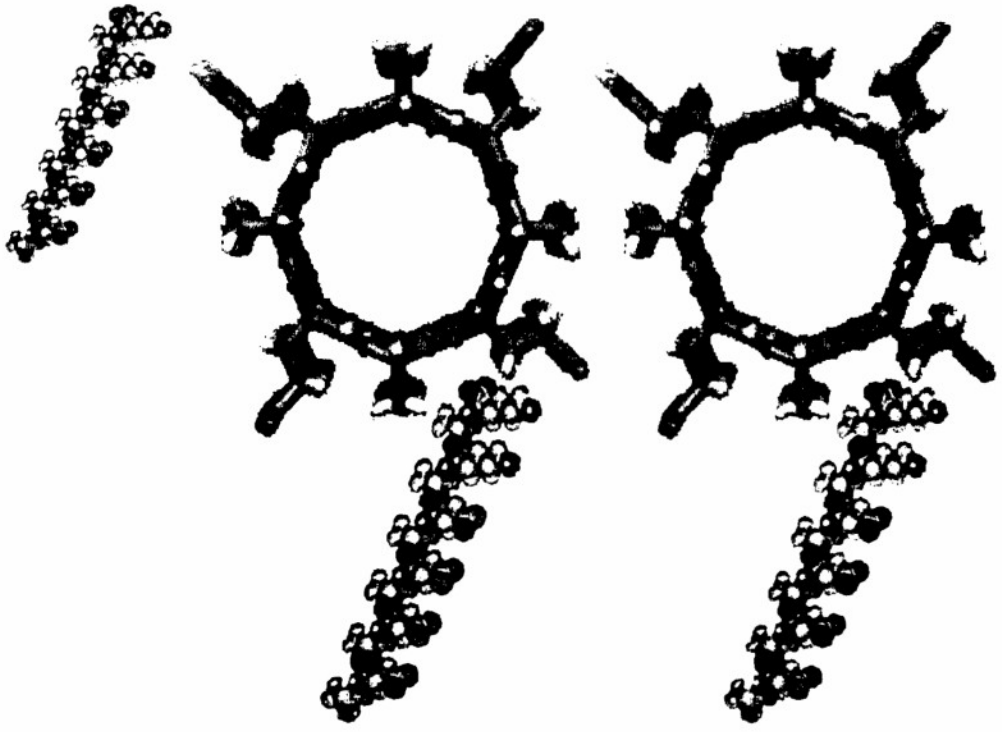
A Multidisciplinary Workshop:

Self-Assembling Peptide Systems in Biology, Medicine and Engineering

Crete, Greece July 1-6, 1999



A Multidisciplinary Workshop: Self-assembling Peptide Systems in Biology, Medicine and Engineering

July 1-6, 1999
Lida Conference Room
Capsis Hotel, Crete, Greece

Program Schedule

Wednesday June 30, 1999 Evening:
Registration and welcome reception: 6:30 p.m. at the **Adonis Terrace** and dinner at the Hotel restaurant 7:30-9:30.

Day 1, Thursday July 1, 1999 Morning: Lida Conference Room

8:30-8:50 Introductory Remarks: Shuguang Zhang, Amalia Aggeli & Neville Boden
Outline of the workshop, its significance and acknowledgment of the sponsors; information about the hotel and the location.

8:50-9:40 ***Keynote Address***: Carleton Gajdusek, Institute of Human Virology, USA
Nucleation of Conformational Change of Proteins in Growth, Development, Learning, Aging and Pathogenesis.

Session 1. The Emerging of Peptide Self-assembling Systems (Chair, Jeff Kelly)

The late Tom Kaiser Alumni Research Activities
9:40-10:20 Peter Lansbury Harvard Medical School, USA
Seeded Protein Polymerization in Alzheimer's Disease and Parkinson's Disease

10:20-10:40 *Coffee Break*

10:40-11:20 Tomikazu Sasaki University of Washington, USA
Nano-scale Patterning of Solid Surfaces with Organic and Peptide Templates
11:20-12:00 Hisakazu Mihara Tokyo Institute of Technology, Japan
Engineering self-assembly of peptides by amphiphilic 2D motifs

July 1 Afternoon: Session 1 Continued (Chair, Peter Lansbury)

2:30-3:10 Jeff Kelly Scripps Research Institute, USA,
Peptidomimetics That Undergo Interfacial Assembly and Nucleate Mineralization: Towards Composite Materials

3:10-3:50 John Taylor Rutgers University, USA
Surface-Binding Properties of a Proline-Rich Domain from Serum Apolipoprotein B

3:50-4:10 *Coffee break*

4:10-4:50 Debbie Kendall University of Conn, USA
Model Signal Peptides: Probes of Molecular Interactions During Secretion
4:50-5:30 Dennis Bong Scripps Institute, USA
Mechanistic Insights in Nonenvelope Viral Transfection

Day 2, Friday July 2, Morning
Session 2. Medicine: Protein Conformational Diseases (Chair, Mihael Polymeropoulos)

8:30-9:10 Christopher Dobson University of Oxford, UK
Mechanism of Amyloid Formation and Its Structural Characterization
9:10-9:50 Michael Polymeropoulos Novartis Pharmacogenetics, USA
Genetics and Biology of Parkinson's Disease

9:50-10:10 *Coffee Break*

10:10-10:50 Susan Lindquist University of Chicago, USA
Chaperone Functions and Prion-Like Aggregation in Yeast
10:50-11:30 Aphrodite Kapurniotu University of Tubingen, Germany
Conformational Transitions of Islet Amyloid Polypeptide in Amyloid Formation
11:30-12:10 Abraham Warshawsky Weizmann Institute
Multifunctional Site-directed Reactive Molecule for Probing Superoxide Dismutase

July 2 Evening
Special Session: Multidisciplinary Research Funding (Chair, Robert Campbell)
8:00-8:30 Robert Campell Army Research Office, USA
Nanosystems and Self-assembly Systems and ARO
8:30-9:00 Eleni Kousvelari NIH, USA
Biomimetics and Tissue Engineering Programs at the National Institute of Health: A Programmatic Perspective
9:00-9:30 Keith Ward Office of Navel Research, USA
Nanobiology and ONR

Day 3, Saturday July 3, Morning

Session 3 : Chemistry and Physics of Self-assembling Peptides (Chair, Amalia Aggeli)

8:30-9:10 Neville Boden University of Leeds, UK
Exploiting Protein-Like Self-Assembly to Engineer Novel Polymers: Tapes, Fibrils and Fibres

9:10-9:50 Ted Atkins University of Bristol, UK
Membrane-like Lamellar structures from Chain-folded Polypeptides

9:50-10:30 Stavros Hamodrakas University of Athens, Greece
Peptide-Analogues of Silkmoth Chorion Protein Segments

10:30-10:50 *Coffee Break*

10:50-11:30 Nick Gay University of Cambridge, UK
Structure and Dynamics of Self-assembling Peptide Filaments

11:30-12:10 Joel Schnur Naval Research Laboratory , USA
The Role of Chirality in the Self-assembly of Biologically-derived Microstructures

12:10-12:50 Hanna Rapaport Caltech, USA
Crystalline Beta-sheet Peptide Monolayers at Interfaces

July 3 Afternoon: Session 3 continued, (Chair, Neville Boden)

2:30-3:10 Sam Stupp Northwestern University, USA
Self-Assembly of Supramolecular Analogues of Proteins

3:10-3:50 Barry Moore, University of Strathclyde, Scotland, UK
Self-assembling monolayers of peptides and liquid-crystalline peptides

3:50-4:10 *Coffee Break*

410-4:50 Sandra Burkett MIT USA
Inorganic-Organic Interfaces in Materials Assembly and Performance

4:50-5:20 Haimanot Bekele Scripps Institute USA
Self-assembling Peptidomimetics Monolayer

Day 4, Sunday, July 4, Morning

Session 4, Protein-Protein Interactions and Peptide Self-Assembly in Biology (Chair, Michael Hecht)

8:30-9:10 Michael Hecht Princeton University, USA
De Novo Proteins from Designed Combinatorial Libraries

9:10-9:50 Sylvie Blondelle Torry Pines Institute, Lo Jolla, USA
Design of self-assembling peptides as catalytic mimics using synthetic combinatorial libraries

9:50-10:30 Yechiel Shai Weizmann Institute, Israel
Peptide-Peptide Interactions in the Membrane: Role in the Folding of Membrane Proteins

10:30-10:50 *Coffee break*

10:50-11:30 Anna Mitraki, Institute of Structural Biology, France
A Self-assembling Peptide from the Adenovirus Coat-fiber Protein

11:30-12:10 Michael Pons University of Barcelona, Spain
Spontaneous Trimerization of Palindromic Peptides

July 4 Evening

7:00-10:00 PM **Aviv Conference Dinner** (Sponsored by the Aviv Foundation)

9:00-9:40 Alexander Rich MIT, USA
Self-assembly of Collagen -- 44 years later

9:40-10:30 Kaiser Alumni
Tom Kaiser: Remembrance of Things Past, Celebration of Things, Present & Future

Day 5, Monday July 5 Morning

Session 5. Biomedical Engineering: Versatility of Self-assembling Peptide Systems (Chairs, Eleni Kousvelari)

8:30-9:10 Dan Urry University of Minnesota, USA
Self-assembling Elastic Peptide-based Polymers as Temporary Functional Scaffoldings for Tissue Restoration

9:10-9:50 Constantinos Sakarellos University of Ioannina, Greece
A new circular helicoid-type, sequential oligopeptide carrier for assembling multiple antigenic peptides

9:50-10:10 *Coffee Break*

10:10-10:50 Shuguang Zhang MIT USA
Self-assembling Oligopeptides in Biology and Biomedical Engineering

10:50-11:30 Ferenc Hudecz, Eötvös University, Budapest, Hungary
Design of polymeric polypeptides for targeting/delivery of bioactive molecules

11:30-12:00 Michael Caplan MIT USA
Controlling of Self-assembling Peptide Materials

July 5 Afternoon, *Session 5: Biomedical Engineering continued (Chair, Masahiko Sisido)*

2:30-3:10 Masahiko Sisido Okayama University, Japan
Novel Peptide Nucleic Acids with Improved Solubility and DNA-Binding Ability

3:10-3:50 Shiroh Futaki Kyoto University, Japan
Extramembrane Control of Transmembrane Peptide Assembly

3:50- 4:10 *Coffee Break*

4:10-4:50 Horst Vogel EPFL, Lausanne, Switzerland
Novel Functional Assays for Screening Ligand-Receptor Interactions

Day 6, Tuesday July 6

Session 6. Morning: Thermodynamics & modeling (Chair, Neville Boden)

8:30-9:10 A.Cooper University of Glascow, UK
Thermodynamics of Protein-protein and Peptide Interactions
9:10-9:50 Bruce Tidor MIT USA
Solvation Effects on Protein Folding, Binding and Design: Exploring the Electrostatic Balance

9:50-10:10 *Coffee Break*

Session 7. Morning: Manufacturing: Large Scale Production (Chair, Klaus During)

10:10-10:50 Klaus During PMB, Koln, Germany
Transgenic Plants for Large Scale Production of Peptides and Proteins
10:50-11:30 Colin McKee PPL Therapeutics, Midlothian, UK
Large Scale Production of Proteins and Peptides from The Milk of Transgenic Animals
11:30-12:10 2 latest breaking news from selected summaries.

Novartis Conference Dinner (Sponsored by Novartis Pharmacogenetics)
7:00-10:00 PM
9:00 –10:00 Carl Knappett University of Cambridge, UK
Ancient Minoan Civilization, Culture and Science in Crete

Preface

The idea of organizing this workshop originated from a series of unexpected events. During a visit with Drs. Amalia Aggeli, Neville Boden and their colleagues at University of Leeds, July 3, 1997, we realized that there are many overlaps in our self-assembling peptide work, crossing several disciplines. In a pizza restaurant, we started to write down, on a postcard of University of Leeds, the names of researchers whose work crosses traditional disciplinary boundaries in the study of self-assembling peptide systems. Many of the names on the speaker list were written on that card. Although we knew many of the people in this field, it was impossible to know everyone who works in the same area. We then did a literature search throughout the internet to add additional names to the list. We spoke and met with many individuals about the workshop, everyone was very enthusiastic. After talking with Dr. Peter Lansbury, we found out that several people we identified from the literature share a common thread-- they are alumni of the late Prof. E. Tom Kaiser who had a profound influence in the field. It is evident from his alumni, many of them have become leaders in many disciplines. We therefore decided to have a special session for the Kaiser alumni. Unfortunately, several Kaiser alumni had to cancel at the last minute due to unexpected events.

The site of Crete to host the workshop was recommended by Dr. Amalia Aggeli. After deciding to have the workshop in Crete, Dr. Aggeli spent several months in Crete doing research and looking for a suitable site. The Capsis Hotel was chosen because it was highly recommended by Dr. Mihael Polymeropoulos and has been frequently used by NATO conferences.

Since this is a new workshop that crosses many disciplines, a significant amount of funding needed to be raised. Dr. Bob Campbell of the US Army Research Office (ARO) strongly supported this idea. The ARO traditionally supports multidisciplinary research. This workshop also models ARO workshops that bring people from many disciplines, biology, engineering and medical science combined with the styles of the Gordon Conference and traditional meetings. Dr. Campbell also suggested to contact the Office of Naval Research (ONR), Defense Advanced Research Project Agency (DARPA) and the European Research Office (ERO). We discussed funding opportunities with Drs. Mihael Polymeropoulos then at NIH, Eleni Kousvelari of NIH/NIDCR, Gerald Fischbach and Connie Atwell of NIH/NINDS. They encouraged us to submit proposals to seek funding. We also received support from the newly established Ellison Medical Foundation that specifically supports research on aging. We thank those who supported this workshop at its early inception, especially, Dr. Susan Lindquist who even postponed a scheduled FASEB meeting from 1999 to 2000 in order to accommodate this workshop. We would like to also thank Drs. Jeff Kelly, Peter Lansbury, Hisakazu Mihara and Michael Hecht for their suggestions and help in organizing this workshop.

We greatly appreciate Drs. Mihael Polymeropoulos, now at Novartis Pharmacogenetics, who is a strong supporter not only fanancially but also scientifically; Jack Aviv, whose Aviv Foundation provided generous support for the workshop; Richard Sprott of the Ellison Medical Foundation, who provided first seed funding; Alan Rudolph of DARPA, Keith Ward of ONR, P.C Huang of NSF and David Root of Corning, who provided crucial support; Burghardt Wittig of Mologen, Berlin, Germany, Stephen Gould of Merck Research Labs, Don Comb of New England Biolabs, Jack DeForrst of Bristol-Myers Squibb, Masanobu Kohsaka of Fujisawa Pharmaceutical, Co. Japan, and the European Peptide Society. We would also like to thank Dr. Arnold Demain of MIT who provided important contacts in seeking industrial support.

Special thanks go to Michael Altman of MIT for his tireless effort to establish and to frequently update the conference webpage. He also designed the cover page of this booklet and the name tags.

Unfortunately, the Kosovo conflict has had a serious negative impact on this workshop. Several people cancelled and there are many fewer participants than expected. The events of April in Athens did not help either. There were times we considered to delay the workshop. We were often in touch with the US Embassy in Greece and other agencies to monitor the Greece situation. We are very pleased that the Kosovo conflict reached a resolution just in time.

We collected one page summaries from most participants. This booklet is not in the format of traditional abstract collections, rather, it provides a summary for general interests and it is meant to facilitate contact and stimulate collaborations. Multidisciplinary research activities always require combined approaches and different thinking that crosses traditional boundaries. As Francis Crick best put, *"In Nature hybrid species are usually sterile, but in science the reverse is often true. Hybrid subjects are often astonishingly fertile, whereas if a scientific discipline remains too pure it usually wilts".*

We hope this will be the first step toward a long lasting series of multidisciplinary activities on self-assembling peptide systems. Enjoy the workshop and Crete.

Shuguang Zhang, Amalia Aggeli and Neville Boden
July, 1999

The Purpose of the Multidisciplinary Workshop

The primary goal of this Multidisciplinary Research Workshop is to advance the emerging field of self-assembling peptide systems. Recently, this field has been actively pursued in several broad research areas. This workshop brings us together from various backgrounds, otherwise we would never meet. This is the first meeting to cover such a broad spectrum of fields, including biology, chemistry, physics, protein science, polymer science, materials science and various engineering disciplines unified under a common theme: *Self-assembling Peptide Systems in Biology, Medicine and Engineering*. It is tremendously exciting to bring biologists, chemists, physicists and various engineers under one roof. A cross-disciplinary meeting will undoubtedly generate a great deal of novel ideas and interdisciplinary collaborations. Biology is reaching the limit of what it can accomplish without the influence of other fields, especially Computer Science, Engineering, and Materials Science. These disciplines will again bring new technologies, techniques and innovations to Biology, allowing Biologists to approach previously unanswerable questions. It is important now, more than ever, that Biologists collaborate with scientists from all fields in order to allow Biology to reach new heights in the next century. It is believed that these unconventional collaborations will produce breakthroughs in many unsolved problems. This workshop will also be an incubator for the development of new technologies. This may evolve into a regular multidisciplinary meeting every two years alternating in the US and Europe.

Biology has asked some fundamental questions about nature and our own being, the origin of species, the development of living systems and the origin of life. In the late 1930's to early 1940's, Biology reached a critical point in its establishment as an experimental scientific discipline. Unlike Physics and Chemistry, Biology had an extensive history as an observational rather than experimental science. Due to the intervention of other fields, most notably Physics, biologists received the technology and techniques necessary to convert biology to an experimental science. Multidisciplinary interaction provided technologies such as X-ray crystallography, as well as the logic and mathematics vital to experimental design and analysis. Once Biology received this initial push from other fields, it quickly came into its own as an experimental science. The analysis of phage genetics in the 1940's was complemented by the determination of DNA structure in 1953, and was quickly followed by an understanding of the flow of genetic information to proteins during the 1960's. This, in turn, directly leads to recombinant DNA technology during the 1970's, and the numerous applications of this technology in the 1980's and 1990's.

Biology once again has determined to accomplish some of the most ambitious endeavors, namely, to decipher the genetic code of the entire human genome and many other genomes at the molecular level, to determine almost all molecular structures of proteins in the next century and to understand function of the individual genes. Biology finds itself, once again, in the same position it was in during the early 1940's. Biology is reaching the limit of what it can accomplish without the influence of other fields, especially Computer Science, Engineering, and Materials Science. These disciplines will again bring new technologies, techniques and innovations to Biology, allowing Biologists to approach previously unanswerable questions. It is important now more than ever, that Biologists collaborate with scientists from all fields in order to allow Biology to reach new heights in the next century.

Peptide self-assembly systems have recently been recognized as an emerging research area. These systems, at first glance, seem completely unrelated to such diverse fields, yet they are intimately relevant. Self-assembling peptide systems represent an enormous diversity of research activities in various disciplines ranging from protein science, biotechnology, peptide chemistry, physics, biomedical engineering, and materials science. For example, in biology, peptide assembly is implicated as the molecular basis of several protein misfolding and aggregation, but at the same time, it is the ideal system to produce new materials for novel engineering applications. Understanding the mechanism of peptide self-assembly will help both disciplines. In the biology, one would find a way to inhibit or delay self-assembly, but in materials science, one would find a way to facilitate the self-assembly process so as to produce stronger materials.

I) Biology: Self-assembling peptide systems can be employed as models to address the fundamental questions of protein folding and protein-protein interactions: namely, how could proteins recognize their partners in order to form complexes or higher order structures in a seemingly improbable environment? Such interactions are often facilitated through self-assembly of protein segments by means of chemical complementarity as well as geometric and structural compatibility. Misfolding and interactions of proteins often lead to aggregates that have significant implications in various fields including medicine and pharmaceutical protein mass-production.

2) Biological Materials Science & Biomedical Engineering: Self-assembling peptide systems are being developed in several areas of engineering for application as potential electrical conducting materials, controlled gel formation, new materials for surfaces modifications and bioadhesives: Self-assembling peptide systems have also been demonstrated for specific cell pattern formation, highly selective and sensitive detection systems, biological scaffolding for tissue engineering, matrices for accelerated wound healing, and a system to encase DNA for targeted gene delivery.

3) Medical Diseases: It is believed that peptide self-assembly of protein fragments cleaved from larger proteins, and changes in protein conformation, play an important role in the clinical symptoms of many aging related diseases. These include several neurological diseases, e.g. Alzheimer's disease, Huntington's Disease, Parkinson's Disease, the Bovine Spongeform Enthepholitis (BSE, also called the Mad Cow's Disease), Scrapie and other Prion related diseases. Understanding the basic mechanism of the self-assembly process may lead to inhibitors that can delay the onset of these aging related diseases.

Research activities at the interface of several disciplines often produce interesting and novel results. It is believed that by bringing the leading researchers from various fields into an intimate workshop, they will look at problems from different angles, and facilitate our understanding of self-assembly phenomena. This workshop will likely become a model for future interdisciplinary meetings that look at the physical basis of problems rather than artificially defined disciplines.

Amalia Aggeli, Mark Bell, Neville Boden, Lisa Carrick, Richard Harding, TCB McLeish, Colin Fishwick

IA Nyrkova and AN Semenov
Center for Self-Organizing Molecular Systems,
University of Leeds, Leeds LS2 0JT, UK

Exploiting Peptide Self-Assembly to Engineer Novel Biopolymers: Tapes, Fibrils and Fibers

Our interest in the SOMS Center is harnessing molecular self-assembly to engineer functional materials. There are many precedents for this in living systems. The lecture will focus on how protein-like self-assembly can be exploited to produce novel biocompatible polymers. We will start by considering the *de novo* design of peptides to self-assemble in solution into micrometer long, semi-flexible, ß-sheet, tape-like polymers with scission energies comparable to the strength of covalent bonds [1]. Next we will discover that these tapes are the elementary unit in a hierarchy of self-assembled structures. For example, we find for some peptides that the tapes aggregate into finite stacks (fibrils) with a periodic left-handed twist about their long axis. These fibrils can, in turn, wrap around each other to form rope-like fibers. These structures are far more rigid than the individual tapes and form nematic states at volume fractions of one tenth of one percent. The fibrils are stabilized by competition between the energy of tape-tape attraction and that of the elastic distortion during incorporation of intrinsically twisted ß-sheet tapes into growing stacks. Similarly, the formation of rope-like fibers is driven by attraction between the edges of ß-sheets in neighboring fibrils. We believe this is a generic model for the behavior of chiral, tape-like polymers. Alzheimer's ß-AP (1-40) peptide will be seen to behave similarly, suggesting the model is relevant to the formation of amyloid fibrils generally.

The SOMS Center itself is an interesting development within the context of the theme of this meeting. It is an Interdisciplinary Research Center set up by the University of Leeds in 1993, to focus on exploiting molecular self-assembly to engineer nanostructured materials and devices. Typically, fifteen postgraduate students and ten postdoctoral research fellows work in the center in collaboration with research staff who come from ten different disciplines across the University. Apart from our work on peptide self assembly [1], there are ongoing activities on synthesis and properties of novel liquid crystals [2], charge transport in discotic liquid crystals [3], designing peptide-based transmembrane ion channels [4], tethered lipid bilayers [5], the behavior of complex fluids on solid substrates [6], and hybrid organic-inorganic devices [7].

[1] Responsive gels formed by the spontaneous self-assembly of peptides into polymeric ß-sheet tapes. A Aggeli, M Bell, N Boden, JN Keen, PF Knowles, TCB McLeish, M Pitkeathly and SE Radford, *Nature*, 1997, **386**, 259-262.

[2] A novel synthesis of calamitic and discotic liquid crystalline derivatives of tetrathiafulvalene (TTF), RA Bissell. N Boden, RJ Bushby, CWG Fishwick, E Holland, B Movaghar and G Ungar, *Chem. Commun.*, 1998, 11-114.

[3] Charge dynamics and recombination kinetics in columnar discotic liquid crystals, N Boden, RJ Bushby, J Clements, K Donovan, B Movaghar and T Kreouzis, *Phys. Rev. B*, 1998, 58, 3063-3074.

[4] Conformation and ion channeling activity of a 27-residue peptide modeled on the single transmembrane segment of the IsK (mink) protein, A Aggeli, ML Bannister, M Bell, N Boden, JBC Findley, PF Knowles and J-C Yang, *Biochemistry,* 1998, 37, 8121-8131.

[5] Attenuated total reflection Fourier transform infrared spectroscopic characterization of fluid lipid bilayers tethered to solid support, YL Cheng, N Boden, RJ Bushby, S Clarkson, SD Evans, PF Knowles, A Marsh, RE Miles, *Langmuir,* 1998, 839-844.

[6] Anchoring and orientational wetting of nematic liquid crystal on self-assembled monolayer substrates, B Alkhairalla, H Allinson, N Boden, SD Evans and JR Henderson, *Phys. Rev. E*, 1999, 59, 3033-3039.

[7] Towards hybrid organic-inorganic devices - An EPSRC Materials Program Network. Please visit our Website: www.chem.leeds.ac.uk/ORCHYD/, & www.chem.leeds.ac.uk/SOMS/soms.html

Michael Altman, Ph.D. Candidate

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, MA 02139-4307
maltman@MIT.EDU

Unusually Stable de novo Designed Dipolar Alpha-helical Peptides

Although protein alpha helices have been well studied and characterized, there is still a lack of consensus concerning the origins of helical stability. We are interested in the role that charged amino acids play in helical stability, and specifically the effects of charge separation. In order to approach this problem, we have de novo designed a class of oligopeptides consisting of four negatively charged aspartate residues on the N-terminus, followed by 2-6 alanine residues, and subsequently four positively charged arginine residues on the C-terminus. These peptides have very large dipole moments against the intrinsic helical dipole and produce very strong alpha helices for their size. The helices are quite stable to changes in pH as well as varying salt concentrations. Each peptide was tested for helical stability through high temperature circular dichroism spectroscopy and comparison of ellipticity at 222nm. Previous results have shown that the helical stability of short peptides generally increases with length, but in our experiments DAR3 with 3 alanines was shown to be more stable than DAR4 and almost as stable as DAR5 with 5 alanines. Molecular modeling revealed that the oppositely charged side chains in DAR3 are within ionic bonding distance, unlike DAR5, and this may account for its unusual stability. Our results show that length and charge separation compete as the major factor in the stability of these short helices, with charge separation dominating DAR3 and length becoming the dominant factor as more alanines are added. These results may have profound implications in the field of protein structure and help to understand the forces behind helical stability.

Michael Altman is a graduate student in Chemistry at MIT. He received his B.S. from MIT. He won the 1999 Rudolph G. Wei Award for Excellence in Research at the Interface of Biology and Engineering and The 1999 Asinari Award for Outstanding Research and Writing in the Life Sciences. He is a member of Sigma Xi, New York Academy of Sciences and Phi Beta Kappa.

Selected Publications:

Zhang, S., Altman, M., Chan, R.K, Lee, P. & Ma, R. (1998) Self-assembling peptides in biology, materials science and engineering. Peptide Science: Present and Future. Kluwer Publishers, Amsterdam 761-768.

Zhang, S. & Altman, M. (1999). Peptide Self-assembly Nanosystems in Materials Science and Engineering. The US Army Research Office Report.

Zhang, S., Yan, L., Altman, M., L=E4ssle, M., Nugent, H., Frankel, F., Lauffenburger, D.A., Whitesides, G.M. & Rich, A. (1999) Biological surface engineering: A simple system for tissue cell pattern formation Biomaterials 20, 1213-1220.

Zhang, S. & Altman, M. (1999) Peptide Self-assembly Systems in functional polymer and engineering. Reactive and Functional Polymers 41, 91-102.

Altman, M., Lee, P. Rich, A. & Zhang. S. (1999) Structural Dynamics of Self-Complementary Ionic Oligopeptides. (Submitted).

Altman, M., Rao, V., Zhang, S. (1999) Unusually Stable de novo Designed Dipolar Alpha-helical Peptides. (In preparation).

MEMRANE-LIKE LAMELLAR STRUCTURES FROM CHAIN-FOLDED POLYPEPTIDES

E. Atkins
Physics Department
University of Bristol
BRISTOL BS8 1TL, UK

The controlled biosynthetic production of sequence-designed artificial proteins is an emerging area of macromolecular science and technology. In general, we seek to understand the factors that govern the relationship between the amino acid sequence and the spatial architecture of the polypeptide molecules. This talk is a review of the controlled creation of crystalline protein structures based on repetitive amino acid sequences that bear a relation to the silk-like - proteins.

The salient features of these crystal structures and textures of a family of sequence-designed monodisperse periodic polypeptides will be presented. The structures have been analysed using X-ray diffraction and supplemented with spectroscopic data. Computer simulated X-ray diffraction patterns are used in the interpretation of the X-ray diffraction results.

In these structures the polypeptide chains fold back and forth in a regular fashion to generate thin (3 -6 nm) lamellae. In the first instance the repetitive amino acid sequences are of the type -[$(AG)_xEG$]-, with integer x from 3 to 6. The data support an antiparallel (ap) -sh e et structure, and all the structures index on orthorhombic sub-lattices similar to that reported for *Bombyx mori* silk fibroin. Structures with polar ap -s h eets and -turns, stacking with the hydrophobic methyl groups of the alanine units in contact, are selected by the X-ray refinement to give the best match with experimental data. The results confirm that the folding periodicity is in-phase with the repetitive amino acid sequences so that the glutamic acid (E) units are confined to the chain-folded lamellar surfaces.

A series of related polypeptides (keeping x = 3) were also investigated, in which the glutamic acid units were replaced successively with: alanine, serine, asparagine, valine, lysine, phenylalanine and tyrosine. Analyses of these crystals enables the effect of changing the amino acid volume on the structure to be investigated. A linear relationship was found the between amino acid volume at the folds and the average intersheet spacing.

The sequence -[$(AG)_3EG(GA)_3EG$]- was also crystallised and the structure studied. This structure enables us to delineate which of two different aspects of the structure dominates: (1) the alanyl-alanyl hydrophobic interactions or (2) the -turn rather thanthe -tu rn in the fold. The results indicate that the -turn is the preferred fold in these chain-folded, stacked pleated ap - sheets structures.

Professor Ted Atkins
Melville Wills Professor of Physics
University of Bristol , UK

My general interests are the three-dimensional shapes of macromolecules using X-ray diffraction, electron microscopy and related physical techniques. I have undertaken studies on biological and synthetic polymers; including polysaccharides, proteins , naturally occurring composites and a whole range of speciaalised synthetics. Currently, I am particularly interested in monodisperse polypeptides, nylons and polyesters.

Haimanot Bekele, Ph.D.

Department of Chemistry and The Skaggs Institute of Chemical Biology.
The Scripps Research Institute, La Jolla, CA 92037
hbekele@scripps.edu

Self-Assembled Peptidomimetics That Nucleate Oriented CdS Nanocrystal.

Haimanot Bekele#, Janos H. Fendler~, and Jeffery W. Kelly#*, #Department of Chemistry and The Skaggs Institute of Chemical Biology. The Scripps Research Institute, La Jolla, CA 92037. Center for Advanced Materials Processing, Clarkson University, P.O. Box 5814, Potsdam, NY 13699-5814

The utilization of an organic surface to nucleate an inorganic crystal growth can be used to control its size, shape, orientation, and morphology, in this case, leading to CdS nanocrystals which are semiconductors. A peptidomimetic composed of the 4-(2 aminoethyl)-6-dibenzofuranpropanoic acid and alpha amino acids including those having side chain linked hydrophobic amides self-assembles into a beta sheet monolayer structure at an air/water interface. The monolayer was characterized using a langmuir-blodgett film balance and by circular dichroism and FT-IR spectroscopies. The carboxylate side chains projecting from the Glu residues in the peptidomimetic monolayer match very closely to the Cd ion placement in the {01.0} face of wurtzite, the face nucleated by monolayer as discerned from electron diffraction studies. The lattice mismatch between the anionic ligands projecting from the monolayer and the {01.0} face of wurtzite in two dimensions appears to be the base for the 25 E size control in the width and length dimension. High resolution TEM studies demonstrate that the nanocrystals are oriented in the same direction implying long range order in the monolayer as well. This approach should be useful for the preparation of composite organic/inorganic materials with applications as catalytic, magnetic, optical, and electronic materials.

Sylvie E. Blondelle, Ph.D.

Biochemistry / Microbiology Department
Torrey Pines Institute for Molecular Studies
sblondelle@tpims.org

Our research is focused on two goals: i) elucidation of the induced conformation and internal packing of peptides and small proteins in aqueous and lipid-like environments to enable the de novo design of functionalized protein-like structures; ii) identification of novel biologically active compounds that exert their action either directly onto microorganisms, or on naturally occurring functional proteins, using synthetic combinatorial libraries (SCLs). The design of protein-like structures has emerged as an important step in the ultimate generation of novel macromolecular receptors and catalysts. We have used the SCL approach to design new protein-like structures that have binding step-based catalytic activity. This approach initially consisted in randomizing specific positions of a self- assembled amphipathic - helical scaffold. The resulting conformationally defined libraries exhibited conformationally dependent decarboxylation effects. The individual complexes identified from these libraries showed a number of structural features that are common to natural proteins. Another study involved the design of novel macromolecules to further our understanding of interconformational processes that occur in a number of neurodegenerative disorders in which soluble, random coil or - helical proteins are converted to insoluble, aggregated -sheet states. We have designed polyalanine- based peptides that undergo conformational changes from monomeric -helices into soluble, highly stable, macromolecular -pleated-sheet complexes having similar structural and physical properties than a number of amyloidogenic peptides. These interconformational processes were found to be environment- and concentration-dependent. In parallel, using a SCL approach, inhibitors of the formation of our model -sheet complexes, as well as of analogs of -amyloid protein, have been generated opening a new strategy for the search for the inhibition or prevention of amyloidogenic peptide formation. The SCL approach was extended to the development of modulators of other biological functions based on blocking the access of proteins to their biological targets. For example, calmodulin (CaM) is involved in the regulation of many cellular calcium-controlled processes and is considered as the primary decoder of Ca2+ information in the cell. Novel D-amino acid hexapeptide inhibitors of CaM activity have been identified from a SCL and were found to inhibit CaM regulation of DNA synthesis in in vivo assays using NKR cells. We also used the SCL approach to develop a wide range of novel broad spectrum or microorganism-specific antimicrobial and/or antifungal compounds. In particular, a number of these compounds exhibit high activity against highly pathogenic strains such as methicillin-resistant Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, and Cryptococcus neoformans. Finally, the development of novel antagonists of HIV-1 replication at the entry level, via blockade of gp120/CD4 interactions and/or gp41 structural changes, represents a potential strategy for HIV-1 treatment. Studies have been initiated to develop novel compounds from SCLs that inhibit fusogenic activity mediated by HIV-1 recombinant glycoprotein using two assay systems mimicking the T-cell line-tropic and macrophage-tropic.

She received Ph.D. in Organic Chemistry from the University of Montpellier, France in 1988 and joined The Scripps Research Institute, La Jolla, CA in 1988 as a postdoctoral research fellow with Dr. Houghten. She then joined the Torrey Pines Institute for Molecular Studies, San Diego, CA, in 1989, where she is currently an Associate Member and leader of the Biochemistry/Microbiology Department.

Dennis T. Bong, Ph.D. Candidate

Department of Chemistry
The Scripps Research Institute
La Jolla, CA 92037
dbong@scripps.edu

Transmembrane passage of non-enveloped viruses or genome translocation must occur through the intermediacy of virus-membrane protein interactions, or direct protein-lipid interactions. We have identified a highly membrane active peptide sequence, γ1, in a nonenveloped RNA animal virus, known as the Flock House nodavirus (FHV). This peptide sequence (with a Met → Nle mutation) represents the N-terminal 21 residues of the 44 residue γ peptide, which is cleaved from the C-terminus of the FHV capsid protein in an autocatalytic proteolysis event that is essential to achieve infectivity. Previous research has revealed the following facts about the Flock House nodavirus: i) infectivity is contingent upon the post-assembly autocatalytic cleavage of the γ peptide, ii) γ is located at the high symmetry axes of the virion, a probable point of rupture and release of RNA, iii) similar cleavage peptide structural motifs have been found in other non-enveloped viruses. We report here a complete description of γ1-lipid interactions using a number of biophysical methods and established the propensity of γ1 for spontaneous partitioning into lipid bilayers concomitant with large increases in membrane permeability.
The coupling of the *in vivo* and structural observations with the results of the present *in vitro* study implicate the cleavage peptide in direct protein-lipid interactions that could effect viral genome translocation across the endosomal membrane. These results support the mechanistic hypothesis of nodaviral infection in which receptor binding destabilizes viral assembly, allowing the delivery of a membrane-disrupting agent (γ peptide) to the target membrane, which subsequently permeablizes the bilayer to the viral genome. Selectivity is hypothesized to be governed by a specific virus-receptor interaction whereas the disruptive action of the cleavage peptide that would allow transmembrane passage of viral genomic material is expected to be relatively non-specific with regard to membranes. Thus, these results validate further *in vivo* examination of the role of the nodaviral cleavage sequences in the transfection process.

Dennis Bong received his B.Sc. in chemistry from U.C. Berkeley where he worked in the labs of Professor K. Peter C. Vollhardt. He then entered the chemistry Ph.D. program at the Scripps Research Institute in 1995 where he conducts research under the direction of Professor M. Reza Ghadiri.

Lorenz Buelow, Ph.D.

MPB Cologne GmbH
Molecular Plant & Protein Biotechnology
Eupener Straße 161, Eingang 60
D-50933 Koeln, Germany
Tel +49 221 4994846, Fax +49 221 4994893
e-mail: buelow@mpb-cologne.com

Use of Immobilized ADH as Biosensor in Combination With Flow-Injection-Analysis For the Detection of Ethanol in Liquids.

Microbial reduction of nitrate in water contaminated by a disposal site.

Induction pattern of the maize GapC4 promoter in potato and resistance breeding directing T4-lysozyme against phytopathogenic bacteria (L. Buelow et al: Induction of the Maize GapC4 Promoter in Transgenic Potato under Anaerobiosis and in *Erwinia carotovora*-Inoculated Tuber Tissue, MPMI 12, 1999, 182-188).

Expression of a self-assembling membrane forming peptide in transgenic plants.

He took examination Abitur at the German school Alexander von Humboldt in Lima, Peru. He then studied medicine at the University of Goettingen, Germany. He also studied biotechnology at the Technical University of Braunschweig, Germany and business administration at the University of Leipzig, Germany. He was PhD student at the Federal Center for Breeding Research on Cultivated Plants, Quedlinburg, Germany in cooperation with the Technical University of Braunschweig, Germany. He is a research scientist at MPB Cologne GmbH, Cologne, Germany.

Sandra L. Burkett, Ph.D.

Department of Materials Science and Engineering, Massachusetts Institute of Technology
77 Massachusetts Avenue, Room 12-009, Cambridge, MA 02139, USA
Tel.: (617) 258-0537, Fax: (617) 258-6936, E-mail: burkett@mit.edu
http://www-dmse.mit.edu/people/faculty/slburkett

Department of Chemistry (2243), Amherst College
P.O. Box 5000, Amherst, MA 01002-5000, USA
Tel.: (413) 542-2730, Fax: (413) 542-2735, E-mail: slburkett@amherst.edu

Sandra Burkett's research at the interface between materials science, chemistry, and biology focuses on fundamental interactions at inorganic–organic interfaces that are relevant to the synthesis and performance of a wide variety of materials systems. For example, solid-state NMR has been applied to elucidate how the geometry of an organic "template" is translated into the structure of a crystallizing zeolite framework. Inorganic–organic interactions are also essential to biomineralization and "biomimetic" synthesis of inorganic materials using macromolecular organic templates. In these fields, the distinguishing feature is the influence of organic species on the structural development of inorganic materials. The inverse scenario, in which an inorganic substrate controls protein and cell adsorption, characterizes biomedical materials issues such as the biocompatibility and activity of orthopedic biomaterials and vaccine and drug delivery devices. Hence, the paradigms developed for biomineralization processes and the techniques used to probe and manipulate inorganic–organic interfaces should be transferable to biomedical materials engineering and in crystal engineering. Current research aims to probe the interfacial, intermolecular interactions that affect the selectivity, conformation, and orientation of adsorbed proteins, with the goal of designing inorganic solid surfaces that exhibit particular activity.

Sandra Burkett received her A.B. in Chemistry from Princeton University in 1990 and her Ph.D. in Chemistry from the California Institute of Technology in 1995. After post-doctoral work with Professor Stephen Mann at the University of Bath (UK), she was appointed as an Assistant Professor of Materials Chemistry in the Department of Materials Science and Engineering at MIT, where she was awarded the John Chipman Career Development Professorship. In July, 1999, she will begin an appointment as an Assistant Professor in the Department of Chemistry at Amherst College. Dr. Burkett has recently received a National Science Foundation CAREER Award and a Whitaker Biomedical Engineering Research Grant. Her current professional memberships include AAAS, American Chemical Society, Materials Research Society, and Society for Biomaterials.

Selected Publications:

1. (a) S. L. Burkett and M. E. Davis, "Mechanisms of Structure Direction in the Synthesis of Pure-Silica Zeolites. I. Synthesis of TPA/Si–ZSM-5." *Chem. Mater.* **7**, *1995*, 920-928.

(b) S. L. Burkett and M. E. Davis, "Mechanisms of Structure Direction in the Synthesis of Pure-Silica Zeolites. II. Hydrophobic Hydration and Structural Specificity." *Chem. Mater.* **7**, *1995*, 1453-1463;

2. S. L. Burkett and M. E. Davis, "Synthetic Mechanisms and Strategies for Zeolite Synthesis." in *Comprehensive Supramolecular Chemistry*, Volume 7, Chapter 16, J. L. Atwood, et al., Eds., Oxford, Pergamon, 1996.

3. S. L. Burkett and S. Mann, "Spatial Organization and Patterning of Gold Nanoparticles on Self-Assembled Biolipid Tubular Templates." *Chem. Commun.*, *1996*, 321-322.

4. S. L. Burkett, S. D. Sims, and S. Mann, "Synthesis of Hybrid Inorganic–Organic Mesoporous Silica by Co-Condensation of Siloxane and Organosiloxane Precursors." *Chem. Commun.*, *1996*, 1367-1368.

5. S. L. Burkett, A. Press, and S. Mann, "Synthesis, Characterization, and Reactivity of Layered Inorganic–Organic Nanocomposites Based on 2:1 Trioctahedral Phyllosilicates." *Chem. Mater.* **9**, *1997*, 1071-1073.

Peter Butko, Ph.D.

Institution: Department of Chemistry and Biochemistry,
University of Southern Mississippi
Hattiesburg, MS 39406, USA
peter.butko@usm.edu

Currently, three projects are being pursued in my laboratory. One is cell membrane permeabilization by a cytolytic toxin from Bacillus thuringiensis var. israelensis which belongs to a family of commercially useful biological insecticides. Surprisingly, its mode of action has not been established. Fluorescence spectroscopy and protein crosslinking are used to investigate the possibilities of the toxin penetration into the lipid bilayer and the toxin aggregation on/in the membrane. Both of these events may influence the toxin's ability to destruct the lipid membrane. The second project is the interaction between the immune system protein C1q and the surface of the Group B Streptococcus bacteria (Streptococcus agalactiae). About 0.1% of newborn infants in the USA are infected with these bacteria. Involvement of C1q in successful clearance of the bacteria by the immune systems is known, but the target molecules that bind C1q on the surfaces of bacteria and phagocytes have not been identified. Enzyme-linked immunosorbent assay and 125I-C1q binding assays are utilized in this project. Our work might not only help to treat the infections, but also provide new insights in the postnatal development of the immune system. The third protein we work on is the fungal amphipathic protein hydrophobin

SELF-ASSEMBLING AMPHIPATHIC FUNGAL PROTEINS HYDROPHOBINS.

Hydrophobins comprise a class of proteins with similar sequence of about 100 amino acids and eight conserved cysteines. These proteins have the remarkable ability to self-assemble on hydrophobic/hydrophilic interfaces into flexible, insoluble membranes. We investigate the nature, kinetics, and thermodynamics of the self-assembly process and the accompanying changes in the hydrophobin conformation. The protein does not contain tryptophan nor tyrosine, but fluorescence labels (covalent, such as IAEDANS, PDAM, or non-covalent, such as bis-ANS) have proved to be good probes for monitoring the hydrophobin's assembly state and conformation. Recently, we were able to clone and express hydrophobin in E. coli. Site-directed mutagenesis can be used to: introduce the fluorescence amino acid tryptophan at crucial sites in the protein sequence; determine the importance of selected regions of the protein for its functioning; probe the role of the conserved cysteines and map the pattern of disulfide bonds in hydrophobin; probe the role, if any, of glycosylation in hydrophobin's self-assembly. The self-assembly process is triggered by boiling or by sonication or shaking the hydrophobin solution. From the technological point of view, it is important to find a more practical and quantitative method for initiating the self-assembly. To this aim, we are testing the effect of temperature, pH, ionic strength, and water activity on the protein conformation and assembly. We believe that hydrophobins will find a large number of potential applications in fields like stabilization of foams and oil dispersions, encapsulation and aqueous delivery of hydrophobic substances, and protective and functional surface modifications or coatings.

I received my undergraduate degree in Physics at Komensky University, Bratislava, Slovakia, and Ph.D. in Biological Sciences/Biophysics at Jozsef Attila University, Szeged, and Hungarian Academy of Sciences, Budapest, Hungary. My formative postdoctoral years were spent building a truly interdisciplinary career; I worked at institutions as diverse as a College of Pharmacy (University of Cincinnati, Cincinnati, OH) and a Department of Physics (Texas Tech University, Lubbock, TX). My main research interest - protein/membrane and protein/protein interactions - crystallized during my employment with Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ont., and Channing Laboratory, Brigham and Women's Hospital/Harvard Medical School, Boston, MA.

Robert J. Campbell, Ph.D.

Associate Director for Biological Sciences
Physical Sciences Directorate
US Army Research Office
Tel: 919-549-4230, FAX: 919-549-4310
campbell@aro-emh1.army.mil

Individual Area of Research Support Interest at Army Research Office:

Biomolecular and Cellular Materials and Processes

(1) Fundamental studies to define structure-function relationships and biochemical interactions for enzymes, receptors and other macromolecules exhibiting mechanisms and properties uniquely relevant to synthetic and degradative pathways of interest to the military, including establishment of the foundations for manipulation and exploitation of biocatalysis, ribosomal and non-ribosomal biosynthesis to enhance permissiveness toward elaboration of useful biomolecular structures and cellular systems designed with "metabolic engineering" in mind. (2) Research to provide insight from nature on novel theoretical principles and mechanisms in sensory and motor function, as well as on materials with extraordinary properties, from biological sources. Includes not only initial molecular events, signal transduction pathways and integrated information processing for the powerful sensing capabilities exhibited in the biological world, but also self-assembly processes, hierarchical structure formation, and functional characterization of biomolecular materials such as those with potential "biomimetic" utility for nanometer scale fabrication or for energy and information transfer, among other possibilities.

Robert Campbell obtained his B.S. Chemistry/Biology, City College of New York, CUNY; and received a Ph.D. Pharmacology, State University of New York, Downstate Medical Center. He then was a post-doctoral fellow/research associate University of Michigan; faculty University of Southern California; program scientific officer Biological Sciences, Office of Naval Research.

He was at Army Research Office, Chief, Pharmacology Branch, Army European Research Office (ERO), Chief, Chemistry and Biology Branch Deputy Director and then Director. He has been at the Army Research Office, Chief, Biochemistry and Neurosciences Branch, Army Research Office, Associate Director, Chemical & Biological Sciences Division. He is now Associate Director for Biological Sciences, Physical Sciences Directorate of the Army Research Office. He is members of American Association for the Advancement of Science, Sigma Xi Scientific Society. He has won several wards, including ONR Service Commendation 1982, Army Performance Awards 1984, 87-89, 92-95, 98. Publications in biochemical sciences, physiology, molecular and behavioral neurosciences, biotechnology and biomimetics.

Michael R. Caplan, Ph.D. Candidate

Department of Chemical Engineering, MIT
Cambridge, MA 02139 USA
Caplan@MIT.EDU

Biomaterials, materials that are intended to come into contact with living tissues, are derived from natural and synthetic sources. These materials are constrained in their application by having a limited range of properties or limited biocompatibilty. Oligopeptide, short protein, biomaterials are promising because they may provide a platform with good biocompatibility and a platform that has easily controlled properties. One particular family of oligopeptides, the EAK family, transforms from a viscous solution in water to a gel in some NaCl solutions. Discovered as a fragment of a DNA binding protein called Zuotin, the EAK family of oligopeptides is characterized by alternating hydrophobic and charged amino acid side chains[1]. We have shown that one member of the EAK family, KFE8 (n-FKFKEFKFE-c), forms large two-dimensional sheets of oligopeptide molecules. These sheets combine, upon the addition of a sufficient concentration of NaCl, to form filaments that connect to form a macroscopic network with gel properties[2]. Self-assembly such as this is important because it can be applied to encapsulate cells or drugs for implantation and because it can improve our understanding of physiological and pathological self-assembly processes.

Current research focuses on our ability to control the self-assembly of oligopeptide materials by altering the primary amino acid sequence of the oligopeptide. We have tracked self-assembly by performing rheological tests upon samples equilibrated at various NaCl concentrations. The lowest NaCl concentration at which the oligopeptide forms a gel is termed the critical NaCl concentration, and this is plotted against primary sequence changes to illuminate trends. Sequence changes such as changing the length of the oligopeptide, changing the hydrophobicity of the hydrophobic side chains, and substituting polar side chains for charged will be studied to determine their effect on self-assembly.

Michael Caplan is a Ph.D. candidate in the Department of Chemical Engineering at the **Massachusetts Institute of Technology**. He received his B.S. in Chemical Engineering and his B.A. in Plan II from the University of Texas at Austin in 1996. As part of his research at the University of Texas he provided data for consultations with the Millipore Corporation. He is currently a member of the American Institute of Chemical Engineers, liberal arts and sciences honor society, chemical engineering honor society, engineering honor society, AAAS, and the Society for Biomaterials (secretary of the MIT student chapter).

Selected Publications:

1. Caplan, M.R., Chiang, C.Y., Lloyd, D.R., Yen, L.Y. (1997), Formation of Microporous Teflon® PFA Membranes via Thermally Induced Phase Separation *Journal of Membrane Science* **130**: 219-237.

References:

1. Zhang, S., Holmes, T., Lockshin, C. and Rich, A. (1993) Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. *Proc. Natl. Acad. Sci. USA*, **90**, 3334-3338.

2. Leon, E.J., Verma, N., Zhang, S., Lauffenburger, D.A. and Kamm, R.D. (1998) Mechanical properties of a self-assembling oligopeptide matrix. *J. Biomater. Sci. Polymer Ed.*, **9**, 297-312.

Alan Colman, Ph.D.

PPL Therapeutics, Roslin,
Edinburgh, EH 25 9PP
United Kingdom
Tel.: 0131 440 4777, Fax: 0131 440 4888
e-mail: acolman@ppl-therapeutics.com

PPL Therapeutics specializes in the production of therapeutic proteins and peptides in the milk of transgenic livestock animals. The core technology on which the company was founded in 1987 involves first the construction of a DNA construct comprising the promoter from a milk protein gene (sheep betalactoglobulin) fused upstream of a human structural gene encoding a protein of potential therapeutic importance. The DNA construct is injected into the fertilized zygote of a suitable livestock species (e.g. Sheep, cow, goat) and the injected embryo taken to term in a foster mother of the same species. Resulting transgenic females are then bred and produce the protein in their milk. Some of these transgenic females have produced as much as 30-60g/litre of human protein in their milk. Currently we are in advanced clinical trials with our lead product, alpha 1 protease inhibitor. We produce about 1.5kg per week of this protein to ~99.9999% purity.

The last two years have seen two important new developments at PPL. First, together with the Roslin Institute, we have pioneered the use of nuclear transfer technology to make transgenic animals. This new technology, which we previously used to make Dolly, represents a quantal leap for the biomedical uses of transgenic animals, since for the first time, gene targeting in livestock species is possible. Second, we have found that the mammary gland of certain species are very efficient at amidating the C-termini of protein fusions which present a glycine residue. This has allowed us to suggest a generic, cost effective method of making g/l amounts of peptides (amidated or non amidated) in the milk of transgenic animals where the transgene encodes a foreign milk protein separated by an excisable peptide linker from the C-terminally located peptide of choice.

Colman is currently research director of PPL Therapeutics, a small (~ 230 employees) biotechnology firm based in Edinburgh, Scotland (PPL Ltd.), Blacksburg, Virginia, US (PPL Inc) and New Zealand (PPL NZ) which specialises in the production of foreign (usually human) proteins in the milk of transgenic livestock (particularly cows and sheep), as well as having a broad program in the area of organ transplantation from pigs. Most of the proteins are for therapeutic or nutraceutical use in humans and one (alpha 1 protease inhibitor) is in advanced clinical trials. Alan Colman obtained a BA degree in Biochemistry in Oxford (1971) and a PhD under John Alan Gurdon, a pioneer of the field of nuclear transfer, at the Laboratory of Molecular Biology in Cambridge, UK (1974). After a series of academic appointments in Oxford and Warwick Universities, he became Professor of Biochemistry in the University of Birmingham. The focus of his academic career was the area of eukaryotic protein secretion, with a particular emphasis on the use of frog oocytes and eggs as in vivo test tubes. Along with Ron James (current managing director of PPL) he has been involved with PPL since its inception in 1987, first as part-time research director, becoming full-time (and leaving Birmingham) in 1993. PPL have recently attracted considerable media attention because of their participation in the work that led to Dolly, the world's first sheep cloned from an adult, somatic cell.

References

A Colman (1996) Production of proteins in the milk of transgenic livestock: Problems, solutions, and successes. *American Journal of Clinical Nutrition* 63: 4, S639-S645.

J McWhir, AE Schnieke, R Ansell, H Wallace, A Colman, AR Scott, and AJ Kind (1996) Selective ablation of differentiated cells permits isolation of embryonic stem cell lines from murine embryos with a non-permissive genetic background. *Nature Genetics* 14: 223-226

Schneike A, Kind A, Ritchie, W, Mycock,K, Scott, A, Ritchie, M, Wilmut 1, Colman A and Campbell K (1997) Human Factor IX transgenic sheep produced by transfer of nuclei from transfected fetal oocytes. Science 278: 2130-2133

McKee C Gibson A Dalrymple M Emslie L Garner I Cottingham 1 (1998) Production of Biologically Active Salmon Calcitonin in the Milk of Transgenic Rabbits. *Nat Biotechnology* 7 647-651

Wilmut, I Schneicke, A McWhir J Kind A, Colman A and Campbell K (1999) Nuclear transfer in the Production of Farm Animals in "Transgenic animals in Agriculture" (ed.s Murray et al) CABI Publishing pp. 67-78

Alan Cooper, Ph.D.

Chemistry Department, Glasgow University
Glasgow, G12 8QQ, Scotland, UK
alanc@chem.gla.ac.uk
http://www.chem.gla.ac.uk/~alanc/alanc.html

Thermodynamics of Protein-Protein and Peptide Interactions

Direct measurement of thermodynamic parameters for association/dissociation of proteins and peptides in solution is now routine using isothermal microcalorimetry (ITC) titration and dilution techniques [1,2]. Examples will be given of measurements involving membrane receptors (colicin/porin [3]), protein subunit assemblies (insulin, pyruvate dehydrogenase [4,5]), vancomycin-peptide and synthetic receptors [6,7], and peptide "tapes". Limited kinetic information may also be obtained from calorimetric experiments. Although measurement of thermal data is relatively straightforward, interpretation and rationalisation of the parameters is frustrated by entropy-enthalpy compensation effects. Large variations in enthalpy (ΔH) and entropy (ΔS) of association frequently compensate to give significantly smaller changes in free energy (ΔG), indeed this familiar and ubiquitous homeostatic effect may be of considerable biological advantage in stabilising self-assembling systems against mutation or environmental changes. Nevertheless, the molecular basis for the phenomenon remains something of a mystery. Partly it arises because of the limited ΔG "window" available experimentally, which implies some large-scale apparent correlation of ΔH and ΔS, but this begs the question why ΔH and ΔS might vary so much in the first place. Temperature dependencies may be rationalised in terms of large heat capacity (ΔC_p) effects, which themselves are a manifestation of the thermodynamics of systems comprising multiple, weak (< kT) interactions. Macromolecular conformational dynamics and "quantum confinement" effects can also contribute.

Selected Publications:

1. A.Cooper (1997). *Microcalorimetry of protein-protein interactions.* Methods in Molecular Biology Vol.88: Protein Targeting Protocols, pp.11-22 (ed. Roger A. Clegg, Humana Press 1997).
2. A.Cooper (1999). *Thermodynamics of protein folding and stability.* "Protein: A Comprehensive Treatise", Volume 2, pp. 217-270. (Editor: Geoffrey Allen. JAI Press Inc., Stamford CT, 1999)
3. L.J.A.Evans, A.Cooper & J.H.Lakey (1996). *Direct measurement of the association of a protein with a family of membrane receptors.* J.Mol.Biol. 255, 559-563.
4. M.Lovatt, A.Cooper & P.Camilleri (1996). *Energetics of cyclodextrin-induced dissociation of insulin.* Eur.Biophys.J. 24, 354-357.
5. H.I.Jung, S.J.Bowden, A.Cooper, Y.N.Kalia & R.N.Perham (in preparation). *Entropy-driven protein-protein interactions in the assembly of the pyruvate dehydrogenase multienzyme complex from Bacillus stearothermophilus*
6. D.McPhail & A.Cooper (1997). *Thermodynamics and Kinetics of Dissociation of Ligand-Induced Dimers of Vancomycin Antibiotics.* J.Chem.Soc. Faraday Trans. 93, 2283-2289.
7. R.Xu, G.Greiveldinger, L.E.Marenus, A.Cooper & J.A.Ellman (1999). *Combinatorial library approach for the identification of synthetic receptors targeting vancomycin resistant bacteria.* J.Am.Chem.Soc. 121, 4898-4899.

Alan Cooper, Ph.D.

Chemistry Department, Glasgow University
Glasgow, G12 8QQ, Scotland, UK
alanc@chem.gla.ac.uk
http://www.chem.gla.ac.uk/~alanc/alanc.html

Research interests:

Physical chemistry of biological macromolecules and their interactions; microcalorimetry; protein foams and other novel biomaterials:

- Protein folding, misfolding and aggregation thermodynamics
- Protein-Ligand/Enzyme-Substrate binding
- Protein-Nucleic Acid interaction
- Peptide-Antibiotic interactions (vancomycin)
- Protein-Protein interaction energetics
- Membrane receptor complexes

- **BBSRC/EPSRC UK Center for Biological Microcalorimetry**

- Nematode polyproteins *

- Frog foam protein nests and other biofoam materials *

*(*with Malcolm Kennedy, IBLS)*

Michal Dadlez, Ph.D.

Institution: Institute of Biochemistry and Biophysics.
Polish Academy of Sciences
IBB PAN, Pawinskiego 5A,
02-106 Warsaw, Poland
Fax (48) 39121623
michald@ibbrain.ibb.waw.pl

DISULFIDE KINETICS IN THE DOUBLE MUTANT CYCLE AS A TOOL TO STUDY INTRA- AND INTERMOLECULAR INTERACTIONS IN PEPTIDES.

We developed new approach to detect folding initiation structures in proteins. We were able to show previously that the interaction of some residues in unfolded BPTI, distant from cysteines accelerates by several-fold the native-like pairing of these cysteines during folding. Recently (Zdanowski, K. & Dadlez, M. (1999) J. Mol. Biol. 287, 433-445), by extending our analysis to the double mutant cycle we characterized the folding initiation site BPTI to more detail. We show that the stability of the folding initiation site in BPTI is enthalpy driven, sensitive to chaotropic agents and ionic strength, but insensitive to stabilizing Hofmeister series salt. Our data indicate that a weakly stable native-like beta hairpin, populated at the level of a few per cent of molecules, provides the driving factor for the propagation of proper residue contacts early in folding of this protein. Our results apply to proteins in general, not necessarily disulfide bonded since the cysteines are used here merely as reporter groups. Currently we extend our studies to inter-molecular interactions. By comparing the kinetics of the disulfide formation in a series of aggregation prone peptides and their mutants we are trying to characterize factors responsible for the stability of bi-molecular structures in thermodynamic terms.

Michal Dadlez, Konrad Zdanowski, Agnieszka Jablonowska, Jerzy Dyczkowski. Polish Academy of Sciences The main area of interest of the group is the search for the structures in unfolded polypeptide chains that initiate folding or aggregation. In continuation of the studies of the early steps in the folding of bovine pancreatic trypsin inhibitor (BPTI), which were carried out by M.D. during postdoctoral training in Peter S. Kim's lab in the Whitehead Institute/MIT, Cambridge, MA, USA.

Assunta De Simone

Center for Biomaterials
University of Sheffield
Sheffield, U.K.
Tel: 0114 2229455
chp98amd@sheffield.ac.uk

The aim of my research is to synthesize a new class of block copolymers based on the concept of replacing the hard segments in conventional polyurethane elastomers with polypeptide sequences. This research will rapidly demonstrate the synthesis, and characterize the morphology and mechanical properties of a range of materials based on polyether soft segments and polypeptide hard segments. The polymers will then be converted into integral skin, micro-cellular bandages for application in the treatment of burns and ulcers.

Block copolymers of peptides and polyethers could provide the ideal solution to the requirements of a synthetic skin substitute for use as a micro-cellular bandage. The local molecular structure of the materials controls its biological specificity/compatibility. These will be made of block copolymers of peptides and polyethylene glycol. Low molecular weight polyethylene glycol is non-toxic and can be eliminated from the body by excretion. The sequence of the peptide component will be used to regulate the interaction of the synthetic material with its biological environment.

The macroscopic structure can be built into the material at the processing stage to make an asymmetric foam which will control permeability whereby the upper surface will be water permeable but will prevent bacteria from entering the wound, while the inner surface will have large pores to promote vascularization and natural healing of the wound.

My research is supervised by Professor Hunter and Professor Ryan under the newly established **Center for Biomaterials at the University of Sheffield.**

Professor Hunter supervises research work in a range of projects in the molecular recognition/biomolecular science area:

- synthesis of self-assembled chromophore arrays for controlled manipulation of light and electrons
- synthetic receptors for recognition of small molecule targets
- synthesis of topologically complex molecules
- physical organic studies of the weak intermolecular interactions which control binding and specificity in biology recognition events
- computer modeling of nucleic acid structure

Professor Ryan concentrates on research in processing-structure-property relationships in polymers. He supervises research work in a range of projects:

- synthesis and characterization of block copolymers with novel architecture
- adhesion and adhesives
- processing and properties of polyurethanes
- crystallization in polymers
- phase separation in polymer blends

Mechanism of Amyloid Formation and its Structural Characterisation

Christopher M. Dobson

Oxford Centre for Molecular Sciences, University of Oxford, New Chemistry Laboratory
South Parks Road, Oxford OX1 3QT, United Kingdom

We have been interested for some time in understanding the fundamental principles that govern protein folding. Considerable progress has been made recently in this area, much of it as a result of the development of ideas as to the nature of the energy surface or landscape for a folding reaction. These ideas have arisen from a combination of theoretical analysis and experimental investigation [1]. Of particular importance in the latter has been the concerted application of a wide range of experimental techniques each able to describe aspects of the structural changes taking place during the folding process. NMR spectroscopy has been a key method in this approach because of its ability to provide structural and dynamical information at the level of individual residues [2].

Recently, our research has also focussed on the question of what happens if proteins do not fold correctly, or if they subsequently find themselves in an environment where at least partial unfolding takes place. We have been investigating in particular the nature of protein fibrils of the type associated with amyloidogenic diseases. One system of particular interest to us has been c-type lysozyme. This has been for some time, one of our model systems for studying fundamental aspects of folding, and the discovery that clinical cases of amyloidosis are connected with single point mutations in the lysozyme gene has enabled us to explore the molecular basis of this disease in a well defined model system [3].

This work has recently been extended by the discovery that many proteins not associated with clinical manifestations of disease can form amyloid fibrils in the laboratory under specific conditions [4,5]. This has enabled us to explore the nature of the structure and mechanism of formation of these fibrils in some detail [6]. This talk will report recent results from our laboratory and the significance of these for understanding protein folding and its links with human disease and biological evolution.

References

1. C.M. Dobson, A. Sali and M. Karplus, "Protein Folding: A Perspective from Theory and Experiment", *Angew. Chem. Int. Ed. Eng. 37,* 868-893 (1998).
2. N.A.J. van Nuland, V. Forge, J. Balbach and C.M. Dobson, "Real-time NMR Studies of Protein Folding", *Accts. Chem. Res. 31*, 773-780 (1998).
3. D.R. Booth, M. Sunde,V. Bellotti, C.V. Robinson, W.L. Hutchinson, P.E. Fraser, P.N. Hawkins, C.M. Dobson, S.E. Radford, C.C.F. Blake and M.B. Pepys, "Instability, Unfolding and Aggregation of Human Lysozyme Variants Underlying Amyloid Fibrillogenesis", *Nature 385.* 787-793 (1997).
4. J.I. Guijarro, M. Sunde, J.A. Jones, I.D. Campbell and C.M. Dobson, "Amyloid Fibril Formation by an SH3 Domain", *Proc. Natl. Acad. Sci. USA 95.* 4224-4228 (1998).
5. F. Chiti, P. Webster, N. Taddei, A. Clark, M. Stefani, G. Ramponi and C.M. Dobson, "Designing Conditions for *in vitro* Formation of Amyloid Protofilaments and Fibrils", *Proc. Natl. Acad. Sci. USA 96*, 3590-3594 (1999).
6. J.L. Jiménez, J. I. Guijarro, E. Orlova, J. Zurdo, C.M. Dobson, M. Sunde and H.R. Saibil, "Cryo-Electron Microscopy Structure of an SH3 Amyloid Fibril and Model of the Molecular Packing", *EMBO J. 18,* 815-821 (1999).

Klaus Düring, Ph.D.

MPB Cologne GmbH
Eupener Str. 161, D-50933 Köln
Tel. +49 / 221 / 4994725, Fax +49 / 221 / 4994893
Email: k.during@mpb-cologne.com http://www.mpb-cologne.com

Klaus During studied chemistry at the University of Tubingen (Germany). He made his Ph.D. thesis in the department of Prof. Jeff Schell at the Max-Planck-Institute for Plant Breeding in Cologne (Germany). During this time he worked on the expression and secretion of T4 lysozyme as well as on the expression and assembly of a functional monoclonal antibody in transgenic tobacco (During et al., 1990) which he successfully completed in 1988. After that he concentrated on the biotechnological exploitation of the developed technology for resistance engineering in transgenic plants towards bacteria when staying at the University of Hamburg (Germany) in the group of Prof. Horst Lorz. There, he developed transgenic potato plants expressing T4 lysozyme for reduced sensitivity to the phytopathogenic bacterium *Erwinia carotovora* (During et al., 1993). A further project aimed at the development of monoclonal antibodies inhibiting pectolytic enzymes of *Erwinia carotovora* which are the major pathogenicity factors. In 1993 Klaus During became head of the Institute for Breeding Methods in Vegetables of the Federal Center for Breeding Research on Cultivated Plants in Quedlinburg (Germany), a governmental research organization. He continued antibacterial resistance engineering (During, 1996; de Vries et al., 1999) and broadened to resistance to fungal pathogens as well. Promising phytopathological results were obtained for antibacterial as well as for antifungal resistance. This led to the discovery of a new antimicrobial mechanism of T4 lysozyme which is encoded by a short helix-forming amphipathic peptide region in its C-terminus.
Further research activities aimed at the identification of suitable promoters for gene expression in the host-pathogen-interaction. Especially, an anaerobically induced promoter from maize has been examined in transgenic potato plants. It is not only induced by reduced oxygen but also by *Erwinia carotovora* (Bulow et al., 1999). Furthermore, it promising for technical post-harvest production of proteins of interest. Molecular Farming was added as a new research area then. A first highly efficient production system was developed for single chain antibodies (scFv) in transgenic potato tubers (Artsaenko et al., 1998). In 1994 Klaus During qualified as a University Lecturer ("Habilitation," Privat-Dozent) at the University of Hamburg (Germany) and moved to the Technical University of Braunschweig (Germany) in 1995. In 1998 he founded a new biotech company, *MPB Cologne GmbH* Molecular Plant & Protein Biotechnology in Cologne (Germany) and left his position in public research to become President & CEO of *MPB Cologne GmbH*. This company is primarily focussed on the bioreactor plant but also exploiting the achieved technological developments in antibacterial and antifungal resistance engineering in transgenic plants. In the first instance *MPB Cologne GmbH* will develop and offer optimized platform technologies and later expand into market-driven production of proteins as well. The core competence is in optimized and adapted expression of foreign proteins in transgenic plants.

Selected Publications:

During, K.; Hippe, S.; Kreuzaler, F.; Schell, J.; Synthesis and self-assembly of a functional monoclonal antibody in transgenic *Nicotiana tabacum*; Plant Molecular Biology 15, 281-293 (1990)
During, K.; Porsch, P.; Fladung, M.; Lorz, H.; Transgenic potato plants resistant to the phytopathogenic bacterium *Erwinia carotovora*; The Plant Journal 3, 587-598 (1993)
During, K.; Genetic engineering for resistance to bacteria in transgenic plants by introduction of foreign genes; Molecular Breeding 2, 297-305 (1996)
Artsaenko, O.; Kettig, B.; Fiedler, U.; Conrad, U.; During, K.; Potato tubers as a biofactory for recombinant antibodies; Molecular Breeding 4, 313-319 (1998)
Bulow, L.; Kohler, U.; Cerff, R.; Hehl, R.; During, K.; Induction of the maize *GapC4* promoter in transgenic potato under anaerobiosis and in *Erwinia carotovora* - inoculated tuber tissue; Molecular Plant-Microbe Interactions 12, 182-188 (1999)
During, K.; Porsch, P.; Mahn, A.; Brinkmann, O.; Gieffers, W.; The non-enzymatic microbicidal activity of lysozymes; FEBS Letters, 449, 93-100 (1999)
De Vries, J.; Harms, K.; Broer, I., Kriete, G., Mahn, A., During, K., Wackernagel, W.; The bacteriolytic activity in transgenic potatoes expressing the T4 lysozyme gene and the effect of T4- and hen egg-white lysozyme on soil and phytopathogenic bacteria; Systematic and Applied Microbiology 22, 280-286 (1999)

Fernando Formaggio, Ph.D.

Organic Chemistry Dept.,
Univ. of Padova - Via Marzolo,
1-35131 Padova, Italy
Tel. +39 049 827 5277; FAX +39 049 8275239;
formaggio@chor.unipd.it, http://www.chemistry.unipd.it/PDCHOR/Biopolymers/biorgan.html

Research Interests: chemistry and conformational properties of peptides containing non natural amino acid residues. In this connection, C–-*tetra*-substituted amino acids are of particular interest for the reasons outlined below.

1. Such amino acids are less reactive than protein amino acids. This makes peptide synthesis more difficult, and leads to the quest for new methods in peptide synthesis. On the other hand, the low reactivity has allowed in many instances the isolation and characterization (even by X-ray crystallography) of otherwise elusive active intermediates in peptide synthesis such as anhydrides, N-carboxyanhydrides, acyl halides, active esters, oxazolones, and acyl azides.

2. C–-tetrasubstituted amino acids enjoy a much reduced conformational freedom as compared to protein (C–-*tri*-substituted) amino acids, and have peculiar conformational properties. Most of the amino acids of this family, when inserted into a peptide chain, are strong promoters of folded and helical conformations such as beta-turns, alpha-helices and 3_{10}-helices. Others give rise to the unusual fully-extended conformation. The research in this field goes along three main lines: a) study of the preferred conformations of such amino acids in homo-oligo-peptides and model compounds in solution (by NMR, FT-IR, CD) and in the crystal state (by X-ray diffraction); b) rational design of conformationally constrained analogs of bioactive peptides with enhanced in vivo stability; c) rational design of rigid peptide scaffolds for molecular recognition or intramolecular interaction.

3. Two amino acids of this family, namely aminoisobutyric acid (Aib) and isovaline (Iva), are present in the sequences of a number of membrane-active, channel-forming natural peptides of fungal origin, called peptaibol antibiotics. A project is currently in progress dealing with the total synthesis of such natural compounds and selected analogs, the study of their conformation (in the crystal state, in solution, and in membrane environment) and their mechanism of action in disrupting membranes.

FORMAGGIO obtained his Chemistry Degree (Laurea) at the University of Padova in 1985; Thesis title: "Synthesis and preferred conformations of peptides derived from aminoisobutyric acid"; Advisor: Prof. Claudio Toniolo. From November 1986 until May 1988 Postdoctoral Research Associate in dr. A. F. Spatola's laboratory (Chemistry Dept., Univ. of Louisville, Kentucky, USA), working on cyclic bioactive peptides with the amide bond replaced by the $-CH_2-NH-$ moiety. From September 1988 until March 1990, he was a teacher of organic chemistry and biochemistry in a high school for dental technicians. From April 1990 to date Researcher at the at the Organic Chemistry Dept. (Univ. of Padova, Italy) working in the group of Prof. C. Toniolo on the synthesis and conformational analysis of peptides containing the sterically hindered C-tetrasubstituted-amino acids. Co-author of 138 scientific publications.

Shiroh Futaki, Ph.D.

Institute for Chemical Research, Kyoto University
Tel: +81-774-38-3211; Fax: +81-774-32-3038
futaki@scl.kyoto-u.ac.jp

The major effort of our research is directed toward the creation of artificial functional proteins such as ion channels using synthetic peptides. We have already developed three approaches for the construction of four-helix bundle proteins composed of individual helices having different amino acid sequences [1-3]. These approaches would be useful for the design of proteins having multiple functional moieties that work concertedly in the molecules. One of these approaches was applied for the assembling of the voltage sensor (S4) segments of the sodium channel to obtain an artificial ion channel protein with rectification [4, 5].

The above approaches are for the control of the peptide assembly by covalent cross-links. In this meeting, we will report on the assembly modulation of a transmembrane peptide by non-covalent interaction through an extramembrane segment. A hybrid peptide was designed that has the alamethicin sequence at the N-terminus as a transmembrane segment and the GCN4-derived leucine-zipper sequence at the C-terminus as an extramembrane segment. Association state of the transmembrane peptides was monitored by observing the ion-channel current of alamethicin. By the introduction of the leucine zipper segment, random assembly of alamethicin was modulated to give a substantially single association state. The assembly was tentatively assigned as a tetramer by the comparison with the open conductance to that of template-assembled alamethicin tetramer [6]. Effects of other extramembrane segments on the assembly modulation of the transmembrane peptide will be also discussed.

Shiroh Futaki received his B.S., M.S., and Ph.D. from Faculty of Pharmaceutical Sciences, Kyoto University. He was appointed as a Research Associate, Faculty of Pharmaceutical Sciences, University of Tokushima. He was then a postdoctoral associate at the Rockefeller University. He was an Associate Professor, Institute for Medicinal Resources, The University of Tokushima and is now an Associate professor, Institute for Chemical Research, Kyoto University.

[1] S. Futaki, K. Kitagawa, Tetrahedron Lett., 35, 1267-1270 (1994)
[2] S. Futaki, et al., Tetrahedron Lett., 36, 5203-5206 (1995)
[3] S. Futaki, et al., Bioorg. Med. Chem., 7, 187-192 (1999)
[4] S. Futaki, et al., Tetrahedron Lett., 38, 7071-7074 (1997)
[5] S. Futaki, Biopolymers (Peptide Science), 47, 75-81 (1998)
[6] A. Matsubara, et al., Chem. Commun., 2069-2070 (1996)

Susanne Gangl, Ph.D.-Candidate

Institute of Theoretical Chemistry and Radiation Chemistry,
Photophysics and Photochemistry
University of Vienna
Research Group of Prof. Gottfried Köhler:,
Althanstrasse 14, UZAII, A-1090 Vienna, AUSTRIA.
Tel: +43-1-31336-1583, Fax: +43-1-31336-790,
susig@asterix.msp.univie.ac.at, http://majestix.msp.univie.ac.at

Our group is studying membrane protein structure and function by combining spectroscopic methods, such as fluorescence, with chemical, biochemical and molecular biological approaches. We are interested in the determining membrane protein structure and the origin of specific lipid-protein and protein-protein interactions.

Our goal is to characterize the process of *alpha-helix formation* in a short peptide containing 13 alanines, 3 lysines and one tryptophan with the sequence AKAAAAKAAAAKAAAAW. Circular dichroism spectroscopy is used to follow thermal unfolding of the peptide; fluorescence resonance energy transfer (FRET) measurements between the donor tryptophan and a dansylgroup give insight into the temperature dependent distance distribution between donor and acceptor.

At present, we are concentrating on the molecular mechanism of selective *protein-membrane interactions.* We aim at understanding the mechanism of membrane penetration and translocation by PGLa, an antimicrobial peptide isolated from frog skin. We use both steady state and time-resolved fluorescence techniques in order to investigate the localization of the membrane-active peptide PGLa, its lateral distribution and effect on the acylchain and the headgroup region of the lipid bilayer. The model membranes used consist of liposomes composed of negatively charged and zwitterionic lipids.

Susanne Gangl is a Ph.D student in the research group "Photochemistry and Photophysics", Prof. Köhler, at the Institute of Theoretical Chemistry and Radiation Chemistry in Vienna, Austria. She received her Magistra of Biochemistry from the University of Vienna.

Susanne Gangl[1], Erich Staudegger[2], Karl Lohner[2], Silvie Blondelle[3], Gottfried Grabner[1] and Gottfried Köhler[1]

[1] *Institut füt Theoretische Chemie und Strahlenchemie, University of Vienna, Austria,*

[2] *Institut für Biophysik und Röntgenstrukturforschung, Österreichische Akademie der Wissenschaften, Austria and*

[3] *Torrey Pines Institute for Molecular Studies, San Diego, CA 92121, USA*

Peptide-induced Perturbation of Membrane Organization

Lipid-peptide interactions are analyzed by fluorescence spectroscopic techniques for a 21-residue antimicrobial peptide from frog skin and model membranes. In particular specific docking of the peptide to the membrane surface and incorporation into the lipid bilayer is followed and the resulting perturbations of the acyl chain order are analyzed.

The location of this cationic peptide within model membranes composed of DMPC and DMPG was investigated by fluorescence emission using a derivative of PGLa carrying a single tryptophan residue. In the case the surface is negatively charged the tryptophan residue is well embedded within the hydrophobic part of the bilayer. Fluorescence resonance energy transfer to 12-(9-anthroyloxy) stearic acid revealed a considerable increase of the spatial separation of the two chromophors at temperatures in the fluid phase thus indicating lateral phase separation induced by the peptide.

Different fluorescence probes reporting specific physico-chemical properties of the lipid bilayer are applied. The change of the shape and position of the emission spectrum of 12-(9-anthroyloxy) stearic acid in the presence of PGLa indicate restricted mobility, decreased microviscosity and a lower degree of water accessibility within the acyl chain region, both in the gel and in the liquid-crystalline phase. A model for the interactions between PGLa and membranes is proposed.

Amyloid-like fibrils from a peptide-analogue of silkmoth chorion proteins

Iconomidou Vassiliki and Hamodrakas Stavros
Department of Cell Biology and Biophysics, Faculty of Biology, University of Athens, Athens 157 01, Greece

The structure and self-assembly properties of a 51-residue peptide-analogue of the central conservative domain of silkmoth chorion (eggshell) proteins were studied in detail. This peptide (ca-peptide) forms amyloid-like fibrils, under a variety of conditions.

The fibrils bind Congo red and thioflavin T. Negative staining, shadowing and cryo-electron microscopy showed that the fibrils are twisted. The average width of the basic unit is ~90 Å and its helical pitch ~400 Å. CD and FT-Raman spectroscopy indicate a β-sheet type of structure. X-ray diffraction patterns from fibers of the ca-peptide, taken with a double-mirror camera, clearly indicate a "rich" cross-β fiber pattern characterized by a meridional reflection at ~4.68 Å and an equatorial reflection at ~10.1 Å.

Modeling studies suggest that a twisted β–sheet of 4-residue β–strands alternating with β–turns is the basic structural motif of the fibrils. The models are similar to the cross-β structure proposed a decade ago for silmoth chorion proteins to dictate the helicoidal architecture of intact, native chorions.

This study may contribute to our efforts to unravel the principles underlying the formation and architecture of silkmoth chorion, a biological structure-polymer with exceptional mechanical and physiological properties and also set the basis for the exploration of new routes for the design and synthesis of novel biocompatible polymers with important properties, and, perhaps, new potential biomedical and industrial applications.

CV of Stavros J. Hamodrakas

Birth: March 10, 1947 **Place:** Kalamata, Greece
Address: Faculty of Biology, Department of Cell Biology and Biophysics, University of Athens, Athens 157.01, Greece
Phone +30-1-7274545 or +30-1-7240091 **FAX** +30-1-7231634
e.mail shamodr@cc.uoa.gr
Position: Assoc. Professor
Education: Ph.D Astbury Dept. of Biophysics, University of Leeds, U.K (1974), B.Sc Physics Dept., University of Athens, Greece (1970)

Research Interests:

1. Study of fibrous and globular protein structure, folding and assembly

Model systems of study include: silkmoth, Drosophila and fish chorions (eggshells), Chironomus fibres, Concanavalin A-saccharide interactions, DHFR-antifolate complexes

2. Prediction of protein structure and function (Development of algorithms for secondary structure prediction, protein modeling, detection of periodic patterns in sequences, membrane structure prediction, receptor detection etc.)
   Methods used: X-ray diffraction and crystallography, transmission & scanning electron microscopy, freeze-fracturing, shadowing, FT-IR, laser-Raman, CD and NMR spectroscopy

Selected publications

Amino acid Periodicities and Their Structural Implications for the Evolutionarily Conservative Central Domain of Some Silkmoth Chorion Proteins **Hamodrakas, S.J.**, Etmektzoglou, Th. and Kafatos, F. C. J. Mol. Biol., (1985) 186, 583-589

Molecular and supramolecular architecture of the Salmogairdneri proteinaceous eggshell during development Papadopoulou, V. K. Galanopoulos and **S. J. Hamodrakas** J. Struct. Biology , (1996) 116, 399-412

The crystal structure of the complex of Concanavalin A with 4'- methylumbelliferyl-α-D-glucopyranoside **S.J. Hamodrakas**, P. N. Kanellopoulos, K. Pavlou and P. A. Tucker J. Struct. Biol., (1997) 118, 23-30

Laser-Raman and FT-IR spectroscopic studies of peptide-analogues of silkmoth chorion protein segments D. C. Benaki, A. Aggeli, G. D. Chryssikos, Y. D. Yiannopoulos, E.I. Kamitsos, E. Brumley, S. T. Case, N. Boden and **S. J. Hamodrakas** Int. J. Biol. Macromol., (1998) 23, 49-59

Is β-pleated sheet the molecular conformation which dictates formation of the helicoidal cuticle? Iconomidou, V. A., Willis, J. H. and **Hamodrakas, S. J.** Insect Biochemistry and Molecular Biology, (1999) 29, 285-292

A novel method for predicting transmembrane segments in proteins based on a statistical analysis of the Swiss Prot database: the PRED-TMR algorithm Pasquier, C.M., Promponas, V.J., Palaios, G., Hamodrakas, J.S. and **Hamodrakas, S.J.** Protein Engineering, (1999) 12, 381-385

Michael H. Hecht, Ph.D.

Department of Chemistry
Princeton University
Princeton, NJ 08544-1009 USA.
Phone: 609-258-2901, Fax: 609-258-6746
hecht@princeton.edu http://www.princeton.edu/~hecht/sindex.html

The research in my laboratory aims to design novel proteins 'from scratch'. This research is motivated both by the desire to understand the basic principles of protein structure and function, and by the expectation that it will soon be possible to construct *de novo* proteins to perform useful functions in biotechnology and medicine.

Our approach to protein design employs combinatorial methods: Rather than design individual protein sequences one-at-a-time, we devise vast libraries of related sequences that are all constrained by essential features of rational design. These libraries of *de novo* protein sequences are then expressed in bacteria from combinatorial libraries of synthetic genes.

Which features of a *de novo* protein sequences must be designed explicitly? To delineate these features, it is instructive to examine natural proteins. Such examination reveals two universal features: Soluble proteins fold into structures that (i) contain an abundance of secondary structure (_-helices and _-strands), and (ii) expose polar side-chains to solvent while burying nonpolar side-chains in the protein interior. Using these two features as central guides for design, we have developed a strategy that facilitates construction of vast combinatorial libraries of de novo protein sequences. By designing the sequence periodicity of polar and nonpolar residues to match the structural periodicity of the desired secondary structure, *de novo* sequences are predisposed to form secondary structures that are amphiphilic and thereby capable of burying nonpolar side chains and exposing polar side chains. Thus, for a _-sheet design, polar and nonpolar residues are designed to alternate, whereas for an___helical design, the sequence periodicity of polar and nonpolar residues is designed to approximate the structural repeat of 3.6 residues/turn. The patterning of polar and nonpolar residues (the 'binary code') is constrained by the design; however, the identities of the polar and nonpolar residues are varied combinatorially. This variability is made possible by the organization of the genetic code: Wherever a nonpolar amino acid is required, we use the degenerate DNA codon NTN to encode Met, Leu, Ile, Val, or Phe; wherever polar amino acids are required, we use NAN to encode Lys, His, Glu, Gln, Asp, or Asn. (N represents mixtures of DNA nucleotides A, G, T & C.)

We have used this binary patterning of polar and nonpolar amino acids to guide the design of several collections of novel proteins. The initial collection was designed to form __helical structures [Kamtekar et al. (1993) *Science* 262, 1680]. Among the resulting __helical proteins, many bind biological cofactors (e.g. heme) [Rojas et al. (1997) *Protein Science* 6, 2512], and several are enzymatically active. More recently we have designed a collection of de novo __sheet proteins that self-assemble into nanofibrils and recapitulate many of the properties of the amyloid structures found in neurodegenerative diseases such as Alzheimer's disease.

Michael Hecht is currently Associate Professor of Chemistry at Princeton University. He received his BA from Cornell University, where he did undergraduate research on protein structure in the laboratory of Harold Scheraga. He received his Ph.D. from MIT, where he did research using genetic approaches to study protein structure and function. He did post-doctoral research on protein design in the laboratory of David and Jane Richardson at Duke University. In 1990 he joined the faculty at Princeton University where he is in the in the Department of Chemistry, and holds affiliated appointments in the Department of Molecular Biology and in the Princeton Materials Institute. He is currently on sabbatical leave (2/99 – 8/99) in the laboratory of Ephraim Katchalsky-Katzir in the Department of Biological Chemistry at the Weizmann Institute of Science in Israel.

Selected Publications:

Kamtekar S, Schiffer JM, Xiong H, Babik JM & Hecht MH (1993) Protein Design by Binary Patterning of Polar and Non-Polar Amino Acids. ***Science*** 262, 1680-1685.

Xiong H, Buckwalter BL, Shieh HM & Hecht MH (1995) Periodicity of Polar and Non-Polar Amino Acids is the Major Determinant of Secondary Structure in Self-Assembling Oligomeric Peptides. ***Proc. Natl. Acad. Sci. (USA)*** 92, 6349-6353.

West MW & Hecht MH (1995) Binary Patterning of Polar and Nonpolar Amino Acids in the Sequences and Structures of Native Proteins. ***Protein Science*** 4, 2032-2039.

Roy S, Ratnaswamy G, Boice JA, Fairman R, McLendon G & Hecht MH (1997) A Protein Designed by Binary Patterning of Polar and Nonpolar Amino Acids Displays Native-like Properties. ***J. Am. Chem. Soc.*** 119, 5302-5306.

Rojas NR, Kamtekar S, Simons CT, McLean JE, Vogel KM, Spiro TG, Farid RS & Hecht MH (1997) De Novo Heme Proteins From Designed Combinatorial Libraries. ***Protein Science*** 6, 2512-2524.

West MW, Wang W, Patterson J, Mancias JD, Beasley JR & Hecht MH (1999) De Novo Amyloid Proteins From Designed Combinatorial Libraries. . ***Proc. Natl. Acad. Sci. (USA (in press).***

Jean-Frangois Hernandez

Institution: Institut de Biologie Structurale, 41, Jules Horowitz, 38027 Grenoble, FRANCE
Other Contact Information: Fax: (33) 4 76 88 54 94
E-Mail Address: hernande@ibs.fr

A Peptide Segment From the Adenovirus Fiber Shaft Forms Amyloid-like Fibers

Working at the Institut de Biologie Structurale (Grenoble, France) in the group headed by Girard Arlaud, I am managing the peptide synthesis activity of the Institute which is mainly devoted to the study of the structure and function of proteins. My principal activity is the chemical synthesis and folding of modules from proteases of the human complement system for structural analysis. A collaboration with Anna Mitraki (IBS) and Mary Luckey (San Francisco University, CA, USA) was recently undertaken, aiming at studying the mechanisms of protein folding and aggregation.

The fiber protein of adenovirus was chosen as a model system. It is the cell attachment part of the virus. It is a homotrimer of 582-residue monomers and consists of three segments: a C-terminal globular head, a shaft, and a short N-terminal tail which anchors the fiber into the virus capsid.
To characterize the fiber shaft, a model peptide consisting of residues 354-395 of the adenovirus serotype 2 fiber has been chemically synthesized. This part of the shaft is adjacent to the head and has been shown to form trimers when associated to the head (residues 396-582) (Hong and Engler, (1996) J. Virol. 70: 7071-7078). The peptide could only be dissolved in 6 M guanidinium chloride. Upon dilution into phosphate buffer pH 7 or acetate buffer pH 4, the peptide readily formed amyloid-like fibrils, that were visualized by electron microscopy as long ribbons of variable length and 37 1 5 E width, with a helical twist along them (collaboration with Rob Ruigrok, EMBL, Grenoble). When concentrated, the peptide fibers coalesced into sheets that were stained with Congo red, exhibiting the green refringence characteristic of amyloid fibrils.
Further studies of the folding and structure of peptide models from the fiber shaft of adenovirus should provide an insight into the formation of amyloid-like fibrils.

Ferenc HUDECZ, Ph.D.

Head of The Research Group for Peptide Chemistry, Hungarian Academy of Sciences{HAS}, Eotvos L. University, Budapest 112, P.O. Box 32, H-1518 Hungary.
Telephone: (361) 2090-555, Fax: (361)2090-602, E-mail:fhudecz@ludens.elte.hu
Web site: http://www.chem.elte.hu/organic/hudecz.htm

Date of Birth: 12th September 1952 **Place of Birth:** Budapest, Hungary

Qualifications:
1977 Diploma in Chemistry, Eotvos L. University.
1980 Ph.D. Eotvos L. University.
1993 D.Sc. Hungarian Academy of Science.
1996 Habilitation, Eotvos L. University.

Position:
Research Group for Peptide Chemistry, HAS, Eotvos L.University. Head 1999-, Professor 1993-1999, Assistant Professor 1989-1993, Research Fellow 1980-1983, Junior Research Fellow 1977-1980.

The Wistar Institute, University of Pennsylvania, Philadelphia, PA USA. Research Associate 1983-1984.

Cancer Research Laboratories, University of Nottingham, UK. Research Fellow 1988-1989.

Department of Microbiology, Kumamoto University, Japan. Visiting Professor 1991-1992.

Fellowships:
1997: The Royal Society; 1993: U.S.-Hungarian Science and Technology Joint Fund; 1992: TEMPUS; 1990: Unilever; 1989: EACR; 1981: EMBO.

Member of:
The American Chemical Society (ACS), European Association for Cancer Research (EACR), The Biochemical Society (UK), European Peptide Society (member of the council 1998-, member of the executive committee 1998-).

Research Interests:

1. Synthesis and conformational analysis of synthetic branched polymeric polypeptides. Antigenicity, pyrogenicity, toxicity, biodistribution, immunomodulatory potential and enzymatic degradation of synthetic branched polypeptides.

2. Design of drug delivery systems using macromolecular carrier: synthesis, structure analysis and stability studies of monoclonal antibodies – (intermediate carrier) – antitumour agent

(daunomycin, methotrexate, boron compounds, hormones) conjugates for targeting.

3. Design of synthetic antigens using peptidic and non-peptidic epitopes for vaccine and diagnostics development. Prediction, synthesis, conformation and conjugation of viral (herpes simplex virus), bacterial (*M.tuberculosis*) and tumour associated (mucin) antigenic determinants to synthetic macromolecular carriers.

Selected References:

1. Hudecz, F.: Design of synthetic branched-chain polypeptides as carriers for bioactive molecules. Anti-Cancer Drugs 6(2):171-193 (1995).
2. Hudecz, F.: Alteration of immunogenicity and antibody recognition of B-cell epitopes by synthetic branched polypeptide carriers with poly(L-lysine) backbone. Biomedical Peptides, Proteins and Nucleic Acids 1:213-220 (1995).
3. Mezo, G., Kajtar, J., Nagy, I., Majar, Zs., Szekerke, M., Hudecz, F.: Carrier design: Synthesis and conformational studies of poly(L-lysine)-based branched polypeptides with hydroxyl groups. Biopolymers, 42: 719-730 (1997).
4. Nagy, I..B., Haro, I., Alsina, A., Reig, F., Hudecz, F: Interaction of branched chain polymeric polypeptides with phospholipid model membranes. Biopolymers, 46: 169-179 (1998).
5. Wilkinson, K.A., Vordermeier, M.H., Wilkinson, R., Ivanyi, J., Hudecz, F.: Synthesis and in-vitro T cell immunogenicity of conjugates with dual specificities : Attachment of epitope peptides of 16 kDa and 38 kDa proteins from *M.tuberculosis* to branched polypeptide. Bioconjugate Chemistry, 9: 539-547 (1998).
6. Sospedra, P., Nagy, I.B., Haro, I., Mestrs, C., Hudecz, F., Reig, F.: Physicochemical behaviour of polylysine[HAV-VP3 peptide] constructs at the air-water interface. Langmuir (1999) (in press).
7. Hudecz, F., Pimm, M.V., Rajnavolgyi, E., Mezo, G., Fabra, A., Gaal, D., Kovacs, A.L., Horvath, A., Szekerke, M.: Carrier design: New generation of polycationic branched polypeptides containing OH groups with prolonged blood survival and diminished *in vitro* cytotoxicity. Bioconjugate Chemistry (1999) (in press).

Robert E. Hughes Ph. D.

Department of Genetics, University of Washington, Box 357360
Seattle, WA 98195-7360 USA. Tel: (206) 616-4523, FAX: (206) 616-3690.
Email: rehughes@u.washington.edu

We are currently interested in developing yeast as a model system for investigating the functions of genes involved in human disease. Our basic experimental premise is that humanized yeast strains can be of utility in elucidating the functions of human disease genes, and that yeast phenotypes (either intrinsic or synthetic) conferred by human gene expression can be used as a basis for genetic and chemical screening.

An area of particular interest is the study of CAG repeat disorders, such as Huntington's disease. A primary cause of Huntington's Disease pathology (and that of other CAG expansion diseases like the Spinocerebellar Ataxias) appears to be mediated by the toxic effects of polyglutamine expression on cellular metabolism. The precise nature of this toxicity is not well understood, however the observation that huntingtin protein containing expanded polyglutamine tracts forms intracellular inclusions suggests that protein misfolding and aggregation may be a unifying pathogenic mechanism for the CAG repeat diseases. We have expressed human huntingtin in yeast and observe allele-specific inclusion formation. We are in the process of developing reporter-gene systems, which will allow us to monitor protein aggregation in yeast growth assays. Such reporter systems have potential use for identifying genetic and chemical modifiers of protein aggregation.

We are also actively studying Alzheimer's Disease genes APP, PS-1 and PS-2 in yeast.

Robert E. Hughes is a Senior Fellow in the laboratory of Dr. Stanley Fields, Howard Hughes Medical Institute and Department of Genetics at the University of Washington. Dr. Hughes received a Ph. D. in biology from Yale University, and a B.S. in microbiology from Rutgers University. He also holds an M A in English literature from Columbia University. Dr. Hughes' work on Huntington's Disease is supported by the Milton Wexler Fellowship of the Hereditary Disease Foundation.

Hiroyuki Kakinuma, Ph.D.

Laboratory of Life Science & Biomolecular Engineering, Japan Tobacco Inc.,
6-2 Umegaoka, Aoba-ku, Yokohama, Japan. Tel: +81-45-972-5901, Fax: +81-45-972-6205,
e-mail: hiroyuki.kakinuma@ims.jti.co.jp

Catalytic antibody (cat Ab) engineering has gained increasing attention for its potential applications such as novel catalysis and medicine. This molecules have often been provided for in vitro molecular evolution to increase the activities by phage-displayed combinatorial library. We have tried to obtain cat Abs efficiently. One is to utilize computational chemistry to design and evaluate transition state analogs for antigens [1]. The other is improving a screening method by a short transition state analogs, which mimic only transition state structural element [2].

We have developed a novel cat Ab which can activate prodrugs to overcome the nonspecific toxicity of anticancer and anti-inflammatory drugs. The interesting features are that a vitamin B_6 derivative is selected for the protecting group (promiety) to mask the virtue of drugs, and the cat Ab, which is generated by immunization for phosphonate transition state analog of vitamin B_6, can particularly recognize the vitamin B_6 moiety rather than drugs. Thus, the cat Ab is expected to be applicable for several drugs which is protected by the vitamin B_6 for the common promoiety.

Hiroyuki Kakinuma is a postdoctoral fellow of the Laboratory of Life Science & Biomolecular Engineering at Japan Tobacco Inc. The project to which he belongs is evolutionary molecular engineering. He received his Ph.D. in carbohydrate chemistry from Tokyo Institute of Technology in Japan. He had experience of exploring novel drugs by synthetic organic chemistry and found an antifungal drug (ER-30346) which have potent and well-balanced activity, and a good safety profile in Eisai Co., Ltd. Now, he is exploring the possibility new position elsewhere.

Acknowledgments

This work is supported by NEDO (New Energy and Industrial Technology Development Organization) as an R&D project of the Industrial Science and Technology Frontier Program.

[1] Hiroyuki Kakinuma, Kazuko Shimazaki, Naoko Takahashi, Kyoko Takahashi, Shigeo Niihata, Yoshiko Aoki, Katsumi Hamada, Hajime Matsushita, Yoshisuke Nishi (1999) Comparison of phosphonate transition state analogs to induce catalytic antibodies and evaluation of key structural factors by an *ab initio* study. *Tetrahedron* **55**, 2559–2572.

[2] Naoko Takahashi, Hiroyuki Kakinuma, Katsumi Hamada, Kazuko Shimazaki, Kyoko Takahashi, Shigeo Niihata, Yoshiko Aoki, Hajime Matsushita, Yoshisuke Nishi (1999) Efficient screening for catalytic antibodies using a short transition-state analogue and detailed characterization of selected antibodies. *Eur. J. Biochem.* **261**, 108–114

Aphrodite Kapurniotu, Ph.D.

Physiological-chemical Institute, University of Tübingen,
Hoppe-Seyler-Str. 4, D-72076 Tübingen, Germany
Tel.: +49-7071-2978781, Fax: +49-7071-293348
afroditi.kapurniotu@uni-tuebingen.de

Our work on the molecular mechanism of islet amyloid polypeptide (IAPP) amyloid formation provides evidence that partially folded IAPP and its self-associated forms are soluble precursors of pancreatic amyloid. Nucleation of IAPP amyloidogenesis by IAPP fibrils leads to a rapid conformational transition into soluble _-sheets followed by formation of insoluble amyloid. The transition occurs via a structured conformeric state with strongly solvent-exposed hydrophobic surface. This state is also populated in the thermal denaturation pathway of IAPP at 45°C and either leads to amyloid formation by prolonged exposure at 45°C or melts into heat denaturated IAPP. Heat denaturated IAPP is more resistant to amyloidogenesis than folded IAPP. In the denaturant-induced denaturation pathway of IAPP the same as above structured population appears at 4.25 M GdnHCl and leads to amyloid formation. The concentration-dependence studies suggest that this amyloidogenic state is a self-associated form of partially unfolded IAPP. We propose that partially unfolded IAPP and its self-associated forms are in a concentration-dependent equilibrium with non-amyloidogenic conformers and may act as early, soluble precursors of amyloid formation. Inhibition of formation of such precursors of amyloidogenesis should assist in the inhibition of amyloid formation and the treatment of type II diabetes and possibly other to amyloidosis-related diseases.

Aphrodite Kapurniotu is a Habilitant in Biochemistry and Group Leader in the Laboratory of Molecular Peptide Research in the Department of Physical Biochemistry at the University of Tübingen, Germany. A. Kapurniotu received her PhD in Chemistry from the University of Tübingen in 1990. She was a postoctoral investigator with Professor John W. Taylor at Rutgers University in New Jersey between 1992-1994 and worked on the synthesis and structure-activity relationships of conformationally constrained calcitonin analogues. She was a senior investigator at the Picower Institute for Medical Research in Long Island, N.Y., with Professors A. Cerami and R. Bucala between 1994-1995 and worked on advanced glycation endproducts and amyloid formation. A. Kapurniotu is a member of the American Chemical Society and the Deutsche Gesellschaft for Biochemie und Molekularbiologie.

Selected Publications:

„Conformational transitions of islet amyloid polypeptide in amyloid formation in vitro“ R. Kayed, J. Bernhagen, N. Greenfield, K. Sweimeh, W. Voelter, & A. Kapurniotu, *J. Mol. Biol.*, 287, 781-796 (1999).

Contribution of advanced glycosylation to the amyloidogenicity of islet amyloid polypeptide (IAPP)“, A. Kapurniotu, J. Bernhagen, N. Greenfield, S. Teichberg, R.W. Frank, Y. Al-Abed, A. Cerami, & R. Bucala, *Eur. J. Biochem.*,251, 208-216 (1998).

„An agent cleaving glucose-derived protein crosslinks in vitro and in vivo“, S. Vasan, X. Zhang, X. Zhang, A. Kapurniotu, J. Bernhagen, S. Teichberg, J. Basgen, D. Wagle, D. Shih, I. Terlecky, R. Bucala, A. Cerami, J. Egan, & P. Ulrich, *Nature (London)*, 382, 275-278 (1996).

Anthony Keefe, Ph.D.

keefe@molbio.mgh.harvard.edu
Harvard Medical School/MGH
Boston, MA, USA

Anthony D. Keefe is in the laboratory of Jack W. Szostak

"mRNA-display" is a method developed in Jack Szostak's research group in which up to 1014 different protein molecules can be individually tagged by their encoding mRNAs (1). This linkage is accomplished by chemically attaching puromycin to the 3' end of the mRNAs in a library. When these RNAs are translated in vitro, the nascent proteins become covalently linked to their encoding mRNAs via this puromycin. These chimeric molecules can then be partitioned by employing selection schemes for binding or catalytic properties.

The Szostak laboratory uses this technique to probe the density and distribution of functional sequences within polypeptide sequence space. In particular, we are interested in the following questions: what fraction of proteins from a random sequence library can accomplish a given function (binding or catalysis)? Can libraries be designed so as to increase the frequency of functional molecules? How many and what kinds of mutations are required to improve or change a protein's biochemical activity?

Anthony Keefe is a post-doctoral fellow in the Szostak lab, and is currently working with libraries composed of random sequence polypeptides with a view to finding those which can bind to ATP. Prior to this he worked in the field of the origin of life, both within the exobiology branch at NASA Ames Research Center, and as a postdoctoral fellow in the laboratory of Stanley Miller of the Urey-Miller spark discharge synthesis of amino acids and other biomolecules (2). Prior to this he undertook research for his Ph.D. at Birmingham University, UK, in the field of redox-active supramolecular chemistry.

(1) Roberts, R.W. & Szostak, J.W. PNAS 94, 12297-12299, (1997).
(2) Keefe, A. D., Miller, S. L. and Newton, G. L., Nature 373, 683-685, (1995).

Jeffery W. Kelly, Ph.D.

Department of Chemistry and The Skaggs Institute of Chemical Biology.
The Scripps Research Institute, La Jolla, CA 92037
Tel: 619-784-9605, FAX: 619-784-9610
jkelly@scripps.edu

- The Kelly Laboratory demonstrated that protein conformational changes alone are sufficient to convert the amyloidogenic protein transthyretin into amyloid fibrils *in vitro*. These publications provide strong evidence that amyloid diseases are protein misfolding diseases, where a protein undergoes a conformational change and subsequently self-assembles into an amyloid fibril. The biophysical studies carried out by the Kelly Laboratory on the mechanism of transthyretin amyloid fibril formation not only make transthyretin the best understood amyloidogenic protein, but have also led to a new mechanism-based therapeutic strategy. In addition, these mechanistic studies have led to a model of the amyloidogenic intermediate which is consistent with solution H/D exchange data and diffraction data from transthyretin amyloid fibrils.

- His group was the first to demonstrate that the mutations that predispose patients to early onset transthyretin amyloid disease appear to do so not by altering the normal three dimensional structure, but by destabilizing transthyretin, making the conformational changes that convert the normal fold to the amyloidogenic fold more facile. Furthermore, recent kinetic studies on the most common mutation (V30M and L55P) demonstrate that these mutations lower the kinetic barrier by three orders of magnitude, allowing the protein to adopt the alternative amyloidogenic conformation much faster than the wild type protein can under partial denaturing conditions. Hence, these mutations appear to alter both the thermodynamics and the kinetics predisposing individuals with these mutations to amyloid formation *in vivo*.

The Kelly group has recently published a novel potentially useful therapeutic strategy to treat individuals with transthyretin amyloid diseases. Briefly, several aromatic compounds have been discovered which bind to transthyretin and variants thereof with high affinity and selectivity in human plasma. Based on mechanistic studies *in vitro*, these compounds effectively prevent the conformational changes required for amyloid fibril formation by stabilizing the transthyretin tetramer structure which is not amyloidogenic by several Kcal / mol, which also increases $\Delta G^{\ddagger}$ for the conformational change required for amyloid fibril formation by a similar amount. Two classes of compounds are now under evaluation in a mouse model of transthyretin amyloid disease. An analogous therapeutic strategy should prove useful for other amyloidogenic proteins that have a defined conformation.

Selected Publications:

Ratnaswamy G, Koepf E, Bekele H, Yin H, Kelly JW The amyloidogenicity of gelsolin is controlled by proteolysis and pH. Chem Biol 1999 6:293-304

Lashuel HA, Lai Z, Kelly JW Characterization of the transthyretin acid denaturation pathways by analytical ultracentrifugation: implications for wild-type, V30M, and L55P amyloid fibril formation. Biochemistry 1998 37:17851-17864.

Peterson SA, Klabunde T, Lashuel HA, Purkey H, Sacchettini JC, Kelly JW Inhibiting transthyretin conformational changes that lead to amyloid fibril formation. Proc Natl Acad Sci U S A 1998 95:12956-12960.

Nettleton EJ, Sunde M, Lai Z, Kelly JW, Dobson CM, Robinson CV Protein subunit interactions and structural integrity of amyloidogenic transthyretins: evidence from electrospray mass spectrometry. J Mol Biol 1998 281:553-564.

Kelly JW The alternative conformations of amyloidogenic proteins and their multi-step assembly pathways. Curr Opin Struct Biol 1998 8:101-106.

Kelly JW The environmental dependency of protein folding best explains prion and amyloid diseases. Proc Natl Acad Sci U S A 1998 95:930-932.

Debra Kendall, Ph.D.

Dept. Molecular and Cell Biology, Box U-44
University of Connecticut
Storrs, CT 06269 USA
Tel: 860-486-1891, FAX: 860-486-1784, E-mail: kendall@uconnvm.uconn.edu
Laboratory web site: http://www.sp.uconn.edu/~mcbstaff/kendall/index.htm

The research efforts of my laboratory are aimed at probing the physical properties of peptides and proteins which enhance membrane-associated processes. These include (1) signal peptides which facilitate protein transport through a transient interaction with a membrane; (2) segments of proteins which function as membrane anchors; and (3) polytopic integral membrane proteins which function in signal transduction (e.g. cannabinoid receptor). Our approach to each of these systems has involved experimentally addressing the following questions: First, what are the key structural motifs that are important for biological function? These include identifying the importance of conformation, the pattern of hydrophobic and hydrophilic residues, folding patterns including interhelical associations, and overall topology. Second, for what steps are these structural motifs important (e.g. membrane insertion, translocation, ligand binding, signal transduction)? And third, what are the critical molecular interactions involved in each step and how does a given physical feature enhance that interaction? These questions are examined by generating synthetic peptides by chemical synthesis corresponding to membrane-interactive domains and assessing for degree of hydrophobicity, self-association and secondary structure using circular dichroism. These are then used in direct binding studies with components with which they may interact in carrying out their biological role. Separately, recombinant proteins are generated in which either a few amino acid replacements are made or entirely new structural segments are inserted. By comparing the wild type and mutant activities *in vivo* and in reconstituted systems, we can assess the contributions of functional groups and various structural elements to biological function. The overall goal is to understand the design principles of membrane proteins and how these features facilitate biological processes in hydrophobic environments.

Debra Kendall is a Professor in the Dept. Molecular and Cell Biology and a Member of the Institute of Materials Science at the University of Connecticut. She received her Ph.D. in Biochemistry from Northwestern University and her B.A. in Biochemistry from Smith College. She is a member of the Physiological Chemistry study section at the NIH and a member of the editorial board for the Journal Biological Chemistry.

Selected Publications:

Kim, J. and Kendall, D.A. (1998) Identification of a Sequence Motif that Confers SecB Dependence on a SecB-Independent Secretory Protein *In Vivo*, *J. Bacteriol.* **180**, 1396-1401.

Miller, A., Wang, L. and Kendall, D.A. (1998) Synthetic Peptides Specifically Recognize SecA and Stimulate ATPase Activity in the Absence of Preprotein, *J. Biol. Chem.*, **273**, 11409-11412.

McMurry, J. and Kendall, D.A. (1999) An Artificial Transmembrane Segment Directs SecA, SecB, and Electrochemical Potential Dependent Translocation of a Long Amino-Terminal Tail, *J. Biol. Chem.* **274**, 6776-6782.

Abadji, V., Lucas-Lenard, J., Chin, C. and Kendall, D.A. (1999) Involvement of the Carboxyl Terminus of the Third Intracellular Loop of the Cannabinoid CB1 Receptor in Constitutive Activation of Gs, *J. Neurochemistry* **72**, 2032-2038.

Peter T. Lansbury, Ph.D.

Center for Neurologic Diseases, Brigham and Women's Hospital
77 Avenue Louis Pasteur, HIM 754
Boston MA 02115
Tel. 617-525-5260, Fax: 617-525-5252
lansbury@cnd.bwh.harvard.edu.

The Fibrillization Behavior of A-Synuclein (As) and Two aS Mutants Linked to Early-Onset Parkinson's Disease.

Peter Lansbury is currently Associate Professor of Neurology at the Harvard Medical School and a principal investigator at the Center for Neurologic Diseases at Brigham and Women's Hospital. Peter grew up in Buffalo, N. Y. and received his undergraduate education at Princeton University, receiving his A. B., with honors, in Chemistry in 1980. He obtained his Ph. D. in Organic Chemistry from Harvard University in 1985, working in the laboratory of Prof. E. J. Corey. He subsequently spent a postdoctoral fellowship in the laboratory of the late Prof. E. T. Kaiser at the Rockefeller University, studying peptide chemistry. Lansbury was an assistant, and later associate, professor of Chemistry at the Massachusetts Institute of Technology from 1988 until 1996, when he accepted a position at Harvard. The Lansbury research group has focussed on developing chemical methods and applying chemical ideas and approaches to the understanding of Alzheimer's disease, scrapie, and Parkinson's disease. Professor Lansbury has received several awards, including the National Science Foundation Presidential Young Investigator Award, the Zeneca Pharmaceuticals Excellence in Chemistry Award, and an Alfred P. Sloan Research Fellowship.

Selected Publications:

1. Lansbury, PT (1997). Structural Neurology: Are seeds at the root of neuronal degeneration? Neuron *19*, 1151-1154.
2. Harper, JD, Lieber, CM, and Lansbury, PT (1997). Atomic force microscopic imaging of seeded fibril formation and fibril branching by the Alzheimer's disease amyloid-ß-protein. Chem. Biol. *4*, 951-959.
3. Harper, JD, Wong, SS, Lieber, CM, and Lansbury Jr., PT (1997). Observation of metastable Aß amyloid protofibrils by atomic force microscopy. Chem. Biol. *4*, 119-125.
4. Conway, KAH, J.D.; Lansbury, P.T. (1998). Accelerated *in vitro* fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. Nat. Med. *4*, 1318-1320.
5. Harper, J (1999). Biochem.
6. Lansbury, PT (1999). Evolution of amyloid: What normal protein folding may tell us about fibrillogenesis and disease. Proc Natl Acad Sci USA *96*, 3342-2244.

Hilal A. Lashuel

The Scripps Research Institute, Department of Chemistry
Tel 619-784-9607
hilal@scripps.edu

Peptidomimetic That Forms Cables, Monolayers (or bilayers) or Fibrils Depending on Solution Conditions: Implications for Materials Science and Neurodegenerative Disease.

Hilal A. Lashuel*, Steven R. LaBrenz, Linda Woo*, Louise C. Serpell and Jeffery W. Kelly*. *Department of Chemistry and The Skaggs Institute of Chemical Biology The Scripps Research Institute, 10550 N. Torrey Pines Rd, MB12La Jolla, CA 92037. Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, United Kingdom.

Understanding the mechanism of b-sheet folding induced self assembly is essential for understanding amyloid formation in neurodegenerative diseases and for developing therapeutic strategies toward inhibiting amyloid fibril formation. We have used circular dichroism (CD), Fourier-transform infrared (FT-IR) spectroscopy, electron microscopy (EM), atomic force microscopy (AFM), X-ray fiber diffraction and analytical ultracentrifugation to examine the structure and aggregation properties of an amphiphilic peptidomimetic that self-assembles into a wide range of structural morphologies reminiscent of those observed for several proteins associated with neurodegenerative diseases. The peptidomimetic known as Diac (VTVT)2dmda exists in a monomeric form that exhibits a characteristic random coil circular dichroism signal in water. Incubation for longer periods of time, addition of salt or increasing the pH above 7, leads to increasing b-sheet content due to the spontaneous dimerization of the peptidomimetic, concomitant with aggregation of the peptide to form bilayers, cables and ribbon-like fibrils rich in (-sheet content as discerned by CD, FTIR and X-ray fiber diffraction. Below pH 7 in the presence of salt, the peptidomimetic assembles into highly soluble quaternary structures of different morphologies suggesting that multiple pathways of self-assembly or kinetically dependent conversion is the source of morphological diversity. At pHs > 7 the peptidomimetic assembles mainly into twisted helical fibrils in addition to insoluble aggregates that precipitate out of solution. In addition, the time course evaluation of self-assembly into fibrillar structures by analytical ultracentrifugation and electron microscopy clearly showed sequential stages that defines the self-assembly pathway and yields important details on b-sheet based self-assembly of amyloid peptides and proteins. The similarities observed in the mechanism of aggregation and in the morphology of the fibrils formed by Diac(VTVT)dmda peptidomimetic to those observed for peptides or proteins involved in neurodegenerative diseases, suggests that this peptidomimetic could serve as a model for evaluating the structural and environmental factors that govern beta-sheet based self-assembly into quaternary structures of varying morphologies.

Susan L. Lindquist, Ph.D.

Howard Hughes Medical Institute,
The University of Chicago
5841 S. Maryland Avenue, N-339, MC 1028
Chicago, IL 60637 USA.
Tel: 773-702-8049, FAX: 773-702- 7254,
s-lindquist@uchicago.edu, http://http.bsd.uchicago.edu/~hsplab

Our work supports the hypothesis that a dominant, cytoplasmically-inherited trait in yeast, [PSI+] replicates by a prion-like mechanism. To explain the unusual genetic properties of [PSI+], Wickner proposed it is a conformationally altered form of the translation-termination factor, Sup35, that doesn't function properly in translation and, once established, is self-perpetuating. We provide genetic, cell biological and biochemical evidence in support of this revolutionary hypothesis for the inheritance of a genetic trait. First, in collaboration with Y. Chernoff and S. Liebman we found that the inheritance of [PSI+] depends upon Hsp104, a chaperone protein that alters the conformational state of other proteins. Next, we examined different strains under conditions that induce, maintain, cure, or repress [PSI+]. Whenever [PSI+] is present, Sup35 is mostly insoluble; when [PSI+] is absent, Sup35 is soluble. A Sup35-Green Fluorescent Protein (GFP) fusion analyzed in living cells is rapidly captured by pre-existing Sup35 aggregates in [PSI+] cells, but remains soluble in [psi-] cells. Thus, the conformational state of newly synthesized protein depends upon the conformational state of pre-existing protein, providing a mechanism by which the trait can be inherited by daughter cells from their mothers. In vitro, purified Sup35 forms highly ordered, self-seeded, amyloid-like fibers, providing a plausible molecular mechanism for this mechanism of inheritance.

Susan Lindquist is an Investigator for the Howard Hughes Medical Institute and the Albert D. Lasker Professor of Medical Sciences at The University of Chicago. Dr. Lindquist received her Ph.D. in biology from Harvard University. Her B.A. in microbiology was awarded from the University of Illinois, Champaign-Urbana. She is a member of The Molecular Medicine Society, The American Society for Cell Biology, The Genetics Society of America, The American Society for Microbiology, and the Federation of American Scientists for Experimental Biology. Dr. Lindquist has been elected to the National Academy of Sciences as well as the American Academy of Arts and Sciences and is a Fellow of the American Academy of Microbiology.

Selected Publications:

Patino, M.M., Liu, J-J., Glover, J.R., and Lindquist, S. 1996. Support for the prion hypothesis for inheritance of a phenotypic trait in yeast. Science. 273: 622-626.

Glover, J.R., Kowal, A.S., Schirmer, E.C., Patino, M.M., Liu, J-J., and Lindquist, S. 1997. Self-seeded fibers formed by Sup35, the protein determinant of [PSI+], a heritable prion-like factor of Saccharomyces cerevisiae. Cell. 89: 811-819.

Lindquist, S. 1997. Mad cows meet psi-chotic yeast: The expansion of the prion hypothesis. Cell. 89: 495-498.

DebBurman, S.K., Raymond, G.J., Caughey, B., and Lindquist, S. Chaperone-supervised conversion of prion protein to its protease-resistant form. Proc. Natl. Acad. Sci. USA. 94: 13938-13943.

Glover, J.R. and Lindquist, S. 1998. Hsp104, Hsp70 and Hsp40: a novel chaperone system that rescues previously aggregated proteins. Cell. 94: 73-82.

Zhou, P., Derkatch, I.L., Uptain, S.M., Patino, M.M., Lindquist, S., and Liebman, S.W. 1999. The yeast non-Mendelian factor [ETA+] is a variant of {PSI+], a prion-like form of release factor eRF3. EMBOJ. 18: 1182-1191.

Solomzi Makohliso, Ph.D.

Laboratory for Physical Chemistry of Polymers & Membranes
Swiss Federal Institute of Technology
Lausanne, Switzerland

The complexity of various biological processes observed in vivo, such as tissue morphogenesis or wound healing, are largely based on highly specific receptor/ligand interactions, wherein the ligands involved may consist of as few as three amino acids (e.g. RGD). This relative simplification provides an inspiration and rationale for pursuing synthetic or mimetic analog systems within the scope of realizing novel biomaterials and bioelectronic devices, such as cell-based biosensors/biochips. Our activities are aimed at studying molecular parameters central to the development of suitable interfaces between electrically active cells, such as neurons, and microelectronic substrates in vitro. Analog peptides derived from laminin, a major component of the extracellular matrix, are being investigated for their possible role as chief mediators of the neuron/microelectronics interface. With the aid of techniques such as surface plasmon resonance, FTIR, and impedance spectroscopy we hope to determine a configuration defining an optimal presentation of the analog biomolecules to the target receptors, such that the longevity of the interface and signal transfer is enhanced. In addition, it is hoped that the results of these studies will also provide a platform for engineering novel peptide-based biochip and biosensor technology.

Solomzi Makohliso is a post-doctoral fellow at the Laboratory for Physical Chemistry of Polymers & Membranes, under the directorship of Prof. Horst Vogel, at the Swiss Federal Institute of Technology in Lausanne. He received his Ph.D. in biomaterials from the Swiss Federal Institute of Technology in Lausanne. He received his B.Sc. in Biomedical Engineering and M.Sc. in Medical Science from Brown University, USA. He is a former member of the Society for Neuroscience, Institute of Electrical & Electronics Engineers (IEEE), and American Society for Artificial Internal Organs (ASAIO).

Selected Publications:

(1). S.A. Makohliso, L. Giovangrandi, D. Leonard, H.J. Mathieu, M. Ilegems, P. Aebischer. Application of Tefloon-AF thin films for bio-patterning of neural cell adhesion. Biosensors & Bioelectronics 13(1998) 1227-1235.

(2). S.A. Makohliso, D. Leonard, L. Giovangrandi, H.J. Mathieu, M. Ilegems, P. Aebischer. Surface Characterization of a Biochip Prototype for Cell-Based Biosensor Applications. Langmuir 15(1999) 2940-2946.

Simone Melchionna, Ph.D.

Department of Chemistry, University of Cambridge
Lensfield Road, Cambridge CB2 1 EW, UK
Phone: +44-1223-336350 - Fax : +44-1223-336362
E-mail : melch@theor.ch.cam.ac.uk, melch@lilith.phys.uniroma1.it

In the study of complex macromolecular systems much effort is devoted in using computer simulations to investigate the structural and dynamical properties. In my approach I use primarily the techniques of Molecular Dynamics to simulate large molecular assemblies, of biological as well as material science interest. I have been involved in studying some peptides, such as Microperoxidases, Glutathione and proteins, such as Superoxide Dismutase and Myoglobin. My primary interests have been in connecting the local dynamics and fluctuations with the structural scaffolding of proteins. In particular the glass-like transition of proteins has been investigated in order to understand the physical mechanism underlying the phase space diffusion of proteins, activated at around 200 K and its universality with the protein structure. Another interesting argument studied concerns
the recurrent dynamical spectrum observed in globular proteins. In the last years a molecular dynamics programme has been released to the scientific community in public domain modality, known as DLPROTEIN, of which I am coauthor. The code collects some of the recent theoretical advances that I have contributed to study in their application to biological systems.

I am currently a postdoc in the group of Prof. Hansen in Cambridge. In the group we are interested in applying a new theoretical technique, known as Density Functional Theory, in its classical form, to understand the structural behaviour and thermodynamics of confined fluids, with its main application to the study of ion channels.

Selected Publications:

Melchionna, S., Ciccotti, G., Holian, B.L., ``Hoover NpT dynamics for systems varying in shape and size" Mol.Phys. 78(1993), 533.

Melchionna, S., Barteri, M., Ciccotti, G., ``Molecular dynamics study of microperoxidases in aqueous and non-aqueous solutions" J.Phys.Chem. 100(1996), 19241.

Melchionna, S., Falconi, M., Desideri, A., ``Effect of temperature and hydration on protein fluctuations: molecular dynamics simulation of Cu,Zn superoxide dismutase at six different temperatures" J.Chem.Phys. 108(1998), 6033.

Melchionna, S., Desideri, A., ``On the origin of the low-frequency modes in globular proteins" Submitted to Phys.Rev. E (1999).

Hisakazu Mihara, Ph.D.

Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan, and PRESTO, Japan Science and Technology Corporation (JST)
Phone: 81-45-924-5756, Fax: 81-45-924-5833, e-mail: hmihara@bio.titech.ac.jp, http://www.bio.titech.ac.jp

Amphiphilic secondary structures play important roles in folding and misfolding of peptides and proteins. For example, diseases caused by misfolding of proteins seem to be controlled by the nature of amphiphilicity of _-helix and _-strand. In another point of view, amyloidogenesis of proteins appears to be one of the common natures of polypeptides. We have attempted to design _-to-_ transitional and amyloidogenic peptides by a simplified manner using amphiphilic secondary structures. In the course of this study, we have pointed out the significance of peptide homogeneity and structural complementarity for the peptide self-organized assembly system.

Hisakazu Mihara, Ph.D. Dr. Hisakazu Mihara received his Ph.D. in Chemistry in 1986 at Kyushu University with Professor Nobuo Izumiya. He was a postdoctoral fellow with Professor Emil T. Kaiser at the Rockefeller University from 1986 - 1988. At the Rockefeller University, he had done the synthesis of an *Antennapedia* homeo domain protein by the Kaiser's method and elucidated the sequence-specific DNA binding and conformational properties of the protein. He was appointed as an Assistant Professor in Applied Chemistry at Kyushu Institute of Technology in 1988, and promoted to an Associate Professor in Applied Chemistry at Nagasaki University in 1993. Currently, he has been involved in Department of Bioengineering, Tokyo Institute of Technology, as an Associate Professor from 1995, and a researcher in the national project of PRESTO program of Japan Science and Technology Corporation from 1998. He is a Visiting Professor at Kyoto University and Konan University. He is a member of AAAS, Chemical Society of Japan, the Society of Synthetic Organic Chemistry Japan, the Society of Polymer Science Japan, the Japanese Biochemical Society, and American Peptide Society. He is also a member of organizing committees of Japanese Peptide Society and Forum on Biomolecular Chemistry, CSJ.

Selected Publications:

1. H. Mihara, E. T. Kaiser, A Chemically Synthesized Antennapedia Homeo Domain Binds to a Specific DNA Sequence, *Science*, **242**, 925-927 (1988)
2. H. Mihara, Y Takahashi, Engineering Peptides and Proteins that Undergo _-to-_ Transitions, *Curr. Opin. Struct. Biol.*, **7**, 501-508 (1997)
3. S. Sakamoto, A. Ueno, H. Mihara, Molecular-Assembly of Two-_-Helix Peptide Induced by Haem-Binding, *J. Chem. Soc., Chem. Commun.*, 1073-1074 (1998)
4. H. Mihara, Y Takahashi, A. Ueno, Design of Peptides Undergoing Self-Catalytic _-to-_ Transition and Amyloidogenesis, *Biopolymers*, **47**, 83-92 (1998)
5. Y Takahashi, A. Ueno, H. Mihara, Design of a Peptide Undergoing _-_ Structural Transition and Amyloid Fibrillogenesis by the Introduction of Hydrophobic Defect, *Chem. Eur. J.*, **4**, 2473-2482 (1998)
6. M. Takahashi, A. Ueno, T. Uda, H. Mihara, Design of Novel Porphyrin-Binding Peptides Based on Antibody CDR, *Bioorg. Med. Chem. Lett.*, **8**, 2023-2026 (1998)
7. S. Sakamoto, A. Ueno, H. Mihara, Design and Synthesis of Haem-Binding Peptides. Relationship between Haem-Binding Properties and Catalytic Activities, *J. Chem. Soc., Perkin Trans. 2*, 2395-2404 (1998)

Anna Mitraki, Ph.D.

CNRS CR1 (French National Research Center
Institut de Biologie Structurale
41, rue Jules Horowitz, 38027 Grenoble, France
tel: (33) 4 76 88 41 37, fax: (33) 4 76 88 54 94, E-mail: mitraki@ibs.fr

Dr. Anna Mitraki research focuses on folding, assembly and aggregation of proteins using adenovirus fibers as a model system. This all-beta-sheet protein is the cell attachment organelle of the virus and consists of three parts: a N-terminal tail, which is noncovalently embedded in the virion, a thin shaft carrying 15-amino acid pseudorepeats, and a globular head (or knob) which recognizes the primary cell receptor. Each knob monomer consists of eight beta strands and the overall shape of the trimer resembles a three-blade propeller (Xia *et al., Structure* 2:1259-1270, 1994).

Her group has recently demonstrated that adenovirus fibers carry a stable C-terminal domain, comprising the knob plus five shaft repeats (Mitraki et al., submitted for publication). This stable domain has been cloned and expressed in *E. coli*, and its crystal structure, including the shaft structure, is being solved at 2.4 A° resolution by Mark van Raaij and Stephen Cusack at EMBL Grenoble. The emerging structural information combined with folding studies will certainly bring further insight to the assembly and stabilization mechanisms of this biological fiber.

In order to simulate folding of the shaft we undertook a synthetic peptide approach. 41-amino acid synthetic peptides corresponding to the shaft region of the stable domain form amyloid fibrils *in vitro* and bind Congo red, a specific amyloid-staining dye. We plan to further investigate the structure and the mechanisms of formation of the aggregated material by cryo-electron microscopy and infrared spectroscopy. Thus, this all-beta sheet protein and its peptides could also serve as a model system for the study of the mechanisms of amyloid formation.

She received her Ph.D. Biochemistry, Université Paris-Sud, Orsay, France (1986) with Jeannine Yon, then did Postdoctoral Research with Dr. Jonathan King in the Department of Biology Massachusetts Institute of Technology, Cambridge, MA, U.S.A.

Mitraki, A., Betton, J. M., Desmadril, M. and Yon, J. M. (1987) Quasi -irreversibility in the unfolding - refolding transition of phosphoglycerate kinase induced by guanidine hydrochloride. European Journal of Biochemistry. **163**: 29-34.

Mitraki, A. and King, J. (1989). Protein folding intermediates and inclusion body formation. Bio/Technology 7:690-697.

Mitraki, A., Fane, B., Haase-Pettingell, C., Sturtevant, J. and King, J. (1991). Global suppression of protein folding defects and inclusion body formation. Science, **253**: 54-58.

Mitraki, A., Danner, M., King, J., and Seckler, R. (1993) Temperature-sensitive mutations and second-site suppressor substitutions affect folding of the P22 tailspike in vitro. J. Biol. Chem. 268: 20071-20075

King, J., Haase-Pettingell, C.A. , Robinson, A. , Speed, M., and Mitraki, A. (1996) Thermolabile folding intermediates: inclusion body precursors and chaperonin substrates. FASEB Journal 10: 57-66.

Barry D. Moore, Ph.D.

Dept P&A Chemistry, University of Strathclyde, Glasgow, Scotland
Tel: +44 (0)141 548 2301, Fax:+44 (0)141 548 4822
b.d.moore@strath.ac.uk

The long term aim of the peptide work is the production of synthetic supramolecular structures for use in intelligent molecular based devices. By analogy with biological systems or a semiconductor chip, such devices must display organization of and communication between multiple disparate elements. Systems which can be programmed to respond to specific changes in their environment and adjust their properties or output accordingly would find a plethora of applications in biology, chemistry and engineering. As in Nature, the key to production of complex molecular based systems is self-assembly. The molecular elements involved must contain groups both for directing structural organisation and for performing their ultimate function within the material. To achieve this there is a need to develop multiple and diverse nano-scale molecular building blocks that can be employed in the construction of new types of ordered structures. We have adopted a biomimetic approach based on synthetic peptides. The modular method of peptide synthesis provides a facile route for introducing many different active elements into the same molecule and to explore many different combinations rapidly. Crucially, peptides also allow control over the relative orientation of these multiple elements via their tuneable solution conformations, detailed knowledge of which is an absolute pre-requisite if the building blocks are to be used in the construction of supramolecular structures. For this reason we concentrate mainly on synthetic peptides that can be handled in non-aqueous environments. In the absence of water even relatively small peptides exhibit some well-defined structure (helix, sheet, turn, etc.), allowing the identification of a range of interesting targets. Goals currently being pursued in this area include; peptide based molecular switches which respond to external stimulus by undergoing conformational transitions [1], helical peptides which self-assemble on metal surfaces to form nano-scale circuit boards[2], mesogen substituted peptides where the secondary structure can be used to modify and tune the liquid crystalline texture [3] and peptides as scaffolds for metal centered enantioselective catalysts. Enzymes are increasingly being used to promote stereo-specific and stereo-selective reactions in organic solvents and supercritical fluids. Under these low water conditions they exhibit surprisingly high stability and catalytic activity and provide interesting new test beds for understanding the fundamental mechanisms behind biocatalyzed reactions[4]. The unusual microenvironment can be used to control normally inaccessible parameters such as the hydration level and we have exploited this in studies of the effect of water on protein stability and dynamics[5]. Of particular practical interest is the development of methods for tuning the enantio- and regio-selectivity of biocatalysts in organic solvents. This requires a detailed understanding of the influence of solvent on the thermodynamics of biomolecular recognition and is a current area of research. Low water conditions also have interesting effects if changes to the protonation state of a protein are considered. In aqueous, counterions to acidic or basic groups are generally dissociated but in low dielectric solvents ion-pairing will occur and changes in protonation state require simultaneous exchanges of counterion. This leads to unusual behavior and a requirement for control over parameters such as pH+pCl and pH - pNa. In collaboration with Prof. Peter Halling we have developed solid-state acid base buffers which effectively behave as chemical pH stats and are able to alter and set the protonation state of enzymes in organic solvents [6]. These will allow fundamental studies of electrostatic interactions in enzyme catalysis.

BDM carried out his Ph.D. work at Nottingham, UK, with Prof. J.J Turner, FRS, and Prof. M. Poliakoff developing an early time-resolved (5s-ns) IR spectrometer. Following postdoctoral periods working on organometallic synthesis in Strasbourg and biosensors at the Institute of Biotechnology in Cambridge, he was appointed as a Lecturer in the Dept of Pure & Applied Chemistry at Strathclyde in 1990 and then to Senior Lecturer in 1997.

1) A reversible transition between an alpha-helix and a 3(10) helix in a fluorescence labeled peptide G. Hungerford, M. Martinez-Insua, D.J.S. Birch and B.D. Moore, Angew. Chem. Int Ed. Engl., 1996, 35, 326-329.
2) Self-assembling monolayers of helical oligopeptides on gold with applications in molecular electronics. A. Strong and B.D. Moore J. Mater. Chem. 1999, 9, 1043-1216.
3) Monodisperse liquid crystalline peptides. P.A.G. Cormack, B.D. Moore and D.C.S. Sherrington, J. Mater. Chem. 1997, 7, 1977-1983.
4) Biocatalyst behavior in low-water systems, G. Bell, P.J. Halling, B.D. Moore, J. Partridge and D.G. Rees Trends Biotechnol. 1995, 13,468-473
5) Activity and Mobility of Subtilisin in Low Water Organic Media: Hydration is more important than solvent dielectric. J. Partridge, P.R. Dennison, B. D. Moore and P. J. Halling Biochim. Biophys. Acta 1998, 1386, 79-89.
6) Control of enzyme activity in organic media by solid-state acid-base buffers. E. Zacharis, B.D. Moore and P.J. Halling. J. Am. Chem. Soc. 1997 119, 12396-1239.

Mihael H. Polymeropoulos, M.D.

mihael.polymeropoulos@pharma.novartis.com
Novartis Pharmaceutical Corporation
938 Clopper Road
Gaithersburg, Maryland 20878
Tel: (301) 330-3101, FAX: (301) 330-2108

For the past 40 years, research into Parkinson's disease (PD) has been predominantly the province of epidemiologists interested in pursuing the connection between the disease and environmental factors such as viral infection or neurotoxins. Hereditary influences were actually discounted because of a high monozygotic twin discordance rate found in studies that were later shown to be inadequate and inconclusive. There has recently been a resurgence of interest in investigating hereditary factors in PD when it became more and more apparent that a positive family history was a major risk factor for the disease. Meanwhile, it also became increasingly apparent from neuropathological studies that the common, idiopathic form of Parkinson's disease had, in fact, a pathological correlate, i.e., the existence of Lewy bodies, an eosinophilic cytoplasmic inclusion body, distributed diffusely throughout the substantia nigra, hypothalamus, hippocampus, autonomic ganglia and olfactory tracts. Although candidate gene approaches to linkage in PD families have not been rewarding, a genome wide scan mapped PD to 4q21-23 in one large family with PD with diffuse Lewy bodies, where a candidate gene, alpha-synuclein, resides. This gene encodes a presynaptic protein of which a peptide fragment is known to be a constituent of Alzheimer's disease plaques. The identification of a missense mutation in the alpha-synuclein gene in four independent PD families suggests that at least some fraction of familial PD with diffuse Lewy bodies is the result of an abnormal protein that interferes with normal protein degradation leading to the development of inclusions and ultimately neuronal cell death. There may be common pathogenetic mechanisms involved in alpha-synuclein mutations in PD and beta-amyloid and presenilin gene mutations in Alzheimer's disease.

Mihael H. Polymeropoulos received his M.D. from University of Patras Medical School, Patras, Greece. He was a Guest Researcher and a Visiting Fellow National Cancer Institute. He was also a Resident in Psychiatry at St. Elizabeths Hospital, Washington, D.C.. He was a Visiting Scientist at the Laboratory of Biochemical Genetics, National Institute of Mental Health at Saint Elizabeths Hospital and a Visiting Scientist at the Laboratory of Genetic Disease Research, National Center for Human Genome Research, National Institutes of Health, Bethesda Md. He is currently the Vice President, Pharmacogenetics, Novartis Pharmaceuticals Corp. **Dr. Polymeropoulos' research on Parkinson's disease was highlighted by President Clinton during his State of the Union Address in January, 1998.**

Selected Publications:

1. Polymeropoulos M.H., Lavedan C., Leroy E., Ide S.E., Dehejia A., Dutra A., Pike B., Root H., Rubenstein J., Boyer R., Stenroos E.S., Chandrasekharappa S. Athanassiadou A., Papapetropoulos T., Johnson W.G., Lazzarini A.M., Duvoisin R.C., Di Iorio G., Golbe L.I., Nussbaum R.L. Mutation in the alpha synuclein gene identified in families with Parkinson's disease. Science. 1997 276:2045-2047.

2. Carstea E.D., Morris J.A., Coleman K.G., Loftus S.K., Zhang D., Cummings C., Gu J., Rosenfeld M.A., Pavan W.J., Krizman D.B., Nagle J., Polymeropoulos M.H., Sturley S.L., Ioannou Y.A., Higgins M.E., Comly M., Cooney A., Brown A., Kaneski C.R., Blanchette-Mackie E.J., Dwyer N.K., Neufeld E.B., Chang T.Y., Liscum L., Tagle D.A. Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. Science. 1997. 277:228-231.

3. Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, Harta G, Brownstein MJ, Jonnalagada S, Chernova T, Dehejia A, Lavedan C, Gasser T, Steinbach PJ, Wilkinson KD, Polymeropoulos MH. The ubiquitin pathway in Parkinson's disease. Nature 1998 Oct 1;395(6701):451-2

4. Polymeropoulos M.H., Kerlavage A.R., McCombie W.R., Venter J.C. Complementary DNA Sequencing: Expressed Sequence Tags and Human Genome Project. Science, 1991; 252:1651-1656.

Miquel Pons, Ph.D.

Departament de Química Orgànica. Universitat de Barcelona
Martí i Franquès, 1-11
08028-Barcelona, Spain
Tel: +34934021263 Fax: + 34933397878
e-mail: miquel@guille.qo.ub.es http://www.qo.ub.es

Our work is focussed in the structural aspects of molecular recognition, de novo peptide design, and in the applications of NMR to the study of molecular structure and dynamics. In particular we are developing NMR methodology for the study of ordered or anisotropic systems and for the study of stereochemically and dynamically complex organic molecules.

Our main areas of interest are:
i) NMR methodology.
ii) De novo designed cysteine based cyclic peptide oligomers.
iii) Natural and de novo designed proline rich peptides for biomedical applications
iv) Structural basis for the selectivity of natural and modified peptide inhibitors of potassium channels.
v) Protease resistant peptide based inhibitors of amyloid formation in Alzheimer disease.

Miquel Pons graduated in Chemistry (1979) and in Biology (1986) at the University of Barcelona. He obtained his Ph.D. degree by the University of London in 1983 under the supervision of Prof. Dennis Chapman. Presently he is Associate Professor in the Organic Chemistry Department of the University of Barcelona. He has published more than 70 papers in the fields of NMR and structural chemistry.

Selected References.

Pons, M. ed. "NMR in supramolecular chemistry". Kluwer Academic Publishers, Dordrecht, 1999.

Royo, M., Contreras, MA, Giralt, E., Albericio, F., Pons, M. "An easy entry to a new high symmetry, large molecular framework for molecular recognition and de novo protein design. Solvent modulation of the spontaneous formation of a cyclic monomer, dimer or trimer from a bis-cysteine peptide." J. Am. Chem. Soc., 120, 6639-6650 (1998).

Mogck, O., Pons, M., Böhmer, V., Vogt, W. "NMR-Studies of the reversible dimerization and guest exchange processes of tetra urea calix[4]arenes using a derivative with lower symmetry." J. Am. Chem. Soc. 119, 5706-5712 (1997).

Magrans, J.O., Ortiz A.R.,. Molins, P.H., Lebouille P., Sánchez-Quesada, J., Prados, P., Pons, M., Gago, F., de Mendoza J., "A designed non-peptidic receptor that mimics the phosphocholine binding site of the MCPC603 antibody." Angew. Chem. Int. Ed. Engl., 35, 1712-1175 (1996).

Fernández, I., Ubach, J., Fuxreiter, M., Andreu, J.M., Andreu, D., Pons, M. "Conformation and self association of a hybrid peptide of cecropin A and melittin with improved antibiotic activity". Chem. Eur. J. 2(7) , 180-188 (1996).

García-Echeverría, C., Albericio, .F., Giralt, E., and Pons, M. "Design, Synthesis, and Complexing Properties of (1Cys-1'Cys, 4Cys-4'Cys)-dithiobis(Ac-L-1Cys-L-Pro-D-Val-L-4Cys-NH2). The First Example of a New Family of Ion-Binding Peptides" J. Am. Chem. Soc., 115, 11663-11670 (1993).

Hanna Rapaport, Ph.D.

Division of Chemistry and Chemical Engineering, mail code 210-41,
California Institute of Technology,
Pasadena, CA 91125,
Tel: 626-395-2510, FAX: 626-793-8472,
E-mail: rhanna@caltech.edu

A new class of beta-sheet peptides was designed to form crystalline monolayers at interfaces. These peptides were synthesized and their monolayer films were characterized by surface pressure-area isotherms and by in situ grazing incidence X-ray diffraction (GIXD) using synchrotron radiation. The diffraction data and lattice energy calculations were utilized for the elucidation of the two-dimensional crystal structure. The tendency of the beta-sheet films to bind to different types of solutes was studied. These crystalline beta-sheet films may have a wide spectrum of implications, ranging from a fundamental understanding of the relation between amino acid sequence and secondary structure to the application of peptides as ordered molecular templates for nanotechnology devices.

Hanna Rapaport, Ph.D. Hanna received her Ph.D. in Chemistry of Materials and Interfaces, in 1998, at the Weizmann Institute of Science (Israel) with Prof. Meir Lahav and Prof. Leslie Leiserowitz. In her Ph.D. work she has characterized the structure of membrane-active compounds in model monolayer films at interfaces, by means of grazing incidence X-ray diffraction. Since August 1998 Hanna is a postdoctral fellow with Prof. David A. Tirrell at the California Institute of Technology, studying crystalline peptide architectures at interfaces.

Selected Publications:

1. "Structural characterization of valinomycin and nonactin at the air-solution interface by grazing incidence x-ray diffraction", H. Rapaport, I. Kuzmenko, K. Kjaer, P. B. Howes, W. Bouwman, J. Als-Nielsen, L. Leiserowitz, M. Lahav J. Am. Chem. Soc. 119, 11211 (1997).
2. "Monitoring the nucleation of crystalline films of cholesterol on water and in the presence of phospholipid", S. Lafont, H. Rapaport, G. J. Somjen, A. Renault, P.B. Howes, K. Kjaer, J. Als-Nielsen, L. Leiserowitz, M. Lahav J. Phys. Chem. B 102, 761 (1998).
3. "Crystalline cyclic peptide nanotubes at interfaces", H. Rapaport, H.S. Kim, K. Kjaer, P.B. Howes, S. Cohen, J. Als-Nielsen, M.R. Ghadiri, L. Leiserowitz, M. Lahav J. Am. Chem. Soc. 121, 1186 (1999).
4. "From nucleation to engineering of crystalline architectures at air-liquid interfaces", H. Rapaport, I. Kuzmenko, R. Edgar, M. Berfeld, K. Kjaer, J. Als-Nielsen, R. Popovitz-Biro, I. Weissbuch, M. Lahav, L. Leiserowitz J. Phys Chem. In press.

Alexander Rich, MD

Department of Biology, Room 68-233
Massachusetts Institute of Technology
Cambridge, MA 02139-4307
Tel.(617) 253-4715, FAX(617) 253-8699
Cbeckman@MIT.EDU, , http://web.mit.edu/biology/www/Ar/rich.html

Alexander Rich has worked on fundamentals, largely on the molecular basis of nucleic acid structure and function. His contributions range from important structural studies to the development of techniques which have been widely used in this field of science. His work is often characterized by making initial discoveries which open up a field to further investigations, leading to large developments in that area of research. This was especially true with his discovery of base-specific double helix formation, DNA-RNA hybridization, discoveries in the mechanism of protein synthesis, structural determinations of collagen, tRNA, Z-DNA and protein factors that are important for nucleic acid function.

Rich and his colleagues have made numerous fundamental scientific discoveries that include: the triple helical structure of collagen (1955), the first nucleic acid hybridization reaction: the self-assembly of two unstructured polynucleotides to form a double helix (1956), RNA can form a double helix (1956), nucleic acid triple helix formation (1957), the first DNA-RNA hybridization (1960), that proteins are synthesized on polysomes (1962), the first single crystal structure revealing the RNA double helix at atomic resolution (1973), tRNA was crystallized (1968), formed high resolution crystals (1971), solved the structure showing a chain folding that incorporated the cloverleaf model (1973) and had many unusual base-base tertiary interactions (1974), a left-handed form of the DNA double helix (Z-DNA) was discovered in an atomic resolution X-ray analysis (1979), the structure of DNA complexed to daunomycin, an anti-cancer drug (1980), the first crystal structure of four-stranded DNA from telomere sequences (1992), the structure of the first translational regulator (1995) from T4 bacteriophage, Z-DNA is stabilized by negative supercoiling (1989), especially associated with transcription (1991) and a nuclear mRNA editing enzyme binds tightly to it (1995), a Z-DNA binding domain was found in the editing enzyme (1997) and the crystal structure of the domain complexed to Z-DNA was solved (1999), the first crystal structure of a viral RNA pseudoknot active in ribosomal frameshifting (1999).

He is the William Thompson Sedgwick Professor of Biophysics at MIT. He was the Fairchild Distinguished Scholar, California Inst. of Technology in 1976, Visiting Scientist, Cavendish Laboratory, Cambridge, England, U.K., 1955-56. He was a Research Fellow, Gates and Crellin Laboratories, California Institute of Technology from 1949-54 with Prof. Linus Pauling. He has won numerous awards including: Merck Award, National Medal of Science, Linus Pauling Medal, Lewis S. Rosenstiel Award, James R. Killian Faculty Achievement. Award, Jabotinsky Medal. He is also Member of National Academy of Sciences, Senior Member of Institute of Medicine, Fellow of American Academy of Arts and Sciences. Fellow of AAAS. He was Fellow of Guggenheim Foundation.

Selected Publications (Over 520):

Rich, Alexander & Crick, Francis. Structure of Collagen. *Nature* **176**, 915-916 (1955)

Crick, Francis & Rich, Alexander. Structure of Polyglycine II. Nature **176**, 780-781 (1955)

Rich, Alexander & Crick, Francis. Molecular Structure of Collagen. J. Mol. Biol. **3**, 483-506 (1962)

Schwartz, T., Rould, M., Lowenhaupt, K., Herbert, A., & Rich, A. Crystal Structure of the Z_ Domain of the Human Editing Enzyme ADAR1 Bound to Left-Handed Z-DNA, *Science*, **284**, 1841-1845 (1999)

Su, L., Chan, L. Egli, M., Berger, J. & Rich, A Minor Groove RNA Triplex in the Crystal Structure of a Viral Pseudoknot Involved in Ribosomal Frameshifting, *Nature Structural Biology*, **6**, 285-292 (1999)

Dominik Rünzler, Ph.D. Candidate

Institute for Theoretical Chemistry and Radiation Chemistry,
University of Vienna, Althanstrasse 14, 1090 Vienna, Austria,
phone: +43-1-31336-1583, fax: +43-1-31336-790,
ruenzler@idefix.msp.univie.ac.at, http://majestix.msp.univie.ac.at

The main point of my work are optical spectroscopic investigations of the self-assembly process of crystalline bacterial cell surface (S-layer) proteins. This is done in collaboration with the working group "Molecular Biotechnology and Biomimetic Membranes" of Prof. Margit Sára at the Center for Ultrastructure Research and Ludwig Boltzmann-Institute for Molecular Nanotechnology (Head of Institute: Prof. U. B. Sleytr).

Crystalline bacterial cell surface layers (S-layers) can be found as the outermost cell envelope component of walled bacteria. They are composed of a single protein or glycoprotein species with molecular weights ranging from 40,000 to 200,000. S-layers completely cover the cell surface and can exhibit either oblique (p1,p2), square (p4) or hexagonal (p3, p6) lattice symmetry with spacings of the morpho-logical units in the range of 3-30 nm. Depending on the lattice symmetry one morpho-logical unit consists of up to 6 identical subunits. Most S-layers are 5-15 nm thick. S-layers possess pores of identical size and morphology in the 2 to 6 nm range. Due to their crystalline character functional groups are found in well defined position and orientation. With respect to their inner and outer faces, they are highly anisotropic structures in their topography and physicochemical properties. Isolated S-layer subunits from numerous bacteria have shown the ability to assemble into two- dimensional arrays either in suspension, on solid supports (e.g. silicon, noble metals, glass), at the air/water interface, on lipid films or liposomes.

Because of the preferentially two-dimensional crystalline structure of S-layer proteins it is impossible up to now to obtain a three-dimensional crystal for x-ray-crystallo-graphy. Therefore different methods are applied to understand the mechanism for intersubunit-bonding and to characterize the protein domains responsible for the two dimensional assembly of the subunits.

Optical spectroscopic measurements provide insight into conformational changes of the subunits during assembly.The intrinsic fluorescence of the tryptophan residues is a useful probe reporting the variation of the environment of these aromatic amino acid during folding and assembly.

Dominik Rünzler is Ph.D.-Student in Prof. G. Köhlers research group "Photochemistry and Photophysics" at the Institute for Theoretical Chemistry and Radiation Chemistry and member of the Institute for Space Biophysics (ISB) – a research institute of the Austrian Society for Aerospace Medicine (ASM).

Constantinos Sakarellos, Ph.D.

Research Laboratory of Peptide Chemistry, Department of Chemistry,
University of Ioannina,
PO Box 1186,
45 110 Ioannina, Greece.
Tel: ++30-651-98390 or 98386, FAX: ++30-651-45203 or 45840,
ksakarel@cc.uoi.gr http://liaison.admin.uoi.gr/erg5en.html

Peptide Chemistry-Immunochemistry: Synthesis, Structure, Spectroscopy and Interactions of Biomolecules (Hormones, Inhibitors, Bioactive Peptides, Antigens) and Macromolecules (Enzymes, Antibodies, Micelles, Membranes). Studies on the relations between «Structure-Conformation-Biological Activity». Synthesis of very active analogues (Agonists or Antagonists) with long half life for possible therapeutic applications (Myasthenia Gravis, Leishmaniosis, Autoimmune Rheumatic Diseases) on the basis of the Physicochemical data (NMR, CD, IR, UV, X-Ray, modeling).

The major target in the design of the presented oligopeptide carriers (SOC_n) was to construct an artificial support with structural rigidity and regularity, so that peptide epitopes could be anchored without conformational restrictions and steric hindrances. ^{1}HNMR studies and molecular modeling showed that SOC_n adopt a distorded 3_{10}-helical structure, which allows a favorable orientation of the lysine side chains and therefore of the attached peptides. Conformational analysis by ^{1}HNMR spectroscopy, of the SOC_n-conjugates pointed out that the peptides anchored to SOC_n retain their original «active» conformation, or that the carrier imposes the prevalence of one conformer, confirming thus our initial design. The SOC_n-conjugates, when used as antigens, displayed significant biological reactivity, while the developed immunoassays were sensitive, convenient and reproducible in screening antibody specificities related to autoimmune diseases. It is very probable that the helicoid structure of SOC_n offers an optimal epitope presentation and helps the reconstruction and/or mimicking of the native epitopes. Immunizations with the SOC_n-conjugates generated in animals high titers of antibodies recognizing, in all cases, the immunogen peptide. Depending on the anchored to SOC_n peptide, it was identified either an immune spreading covering various peptide sequences on the protein, as well as the intact protein, or a limited expansion of the B-cell repertoir. Multiple cross-linking of the B-cell receptor by the B-cell epitopes coupled to SOC_n, or uptake by B-cells and presentation to T-cell receptors which cross-react with these B cell epitopes, are two alternative explanations for the mode of action of $(epitope_n)_x SOC_n$ conjugates and the continous secretion of specific antibodies long time after immunization.

Constantinos Sakarelos, Ph.D. Prof. Constantinos Sakarellos, Received his Ph.D. in the Chemistry of Peptides Synthesis in 1970 at the University of Athens with Prof. Leonidas Zervas. He was a postdoctoral fellow with Prof. Evagelos Brikas in the Institut de Biochimie, Universite de Paris-Sud, Orsay, France, 1973-1975 and with Prof. Murray Goodman at the Department of Chemistry and Biochemistry, in the University of California at San Diego, USA, 1975-1977. He has been collaborating with Dr. Serge Fermandjian in the Service de Biochimie, Department de Biologie, Center des Etudes Nucleaires de Saclay, France, 1978-1985 and with Dr. M. Marraud and M.T. Cung in the Laboratoire de Chimie-Physique Macromoleculaire, ENSIC-INPL, CNRS UA 494, Nancy, France, 1986-today. Lecturer in Organic Chemistry, University of Ioannina, 1977-1981. Aggregation in Organic Chemistry, University of Ioannina, 1981. Professor (Agrege) in Organic Chemistry, University of Ioannina 1981-1982. Assistant Professor, University of Ioannina, 1982-1984. Associate Professor, University of Ioannina, 1984-1988. Full Professor in the Section of Organic Chemistry and Biochemistry, University of Ioannina 1988-today. He a member of Greek Chemical Society, Hellenic Biochemical and Biophysical Society, aEuropean Peptide Society, American Peptide Society.

Selected Publications:

1. "Concept and Design of a New Class of Sequential Oligopeptide Carriers (SOC) for Covalent Attachment of Multiple Antigenic Peptides".V. Tsikaris, C. Sakarellos. M.T. Cung. M. Marraud and M. Sakarellos-Daitsiotis. Biopolymers. 38. 291-293 (1996).
2. "Immunoreactivity and Conformation of the P-P-G-M-R-P-P Repetitive Epitope of the Sm Autoantigen" V. Tsikaris, P.G. Vlachoyiannopoulos. E. Panou-Pomonis. M. Marraud. C. Sakarellos. H.M. Moutsopoulos and M. Sakarellos-Daitsiotis, Int. J. Peptide Protein Res., 48, 319-327, (1996).
3. "The PPGMRPP repetitive epitope of the Sm autoantigen: Antigenic specificity induced by conformational changes. Application of the Sequential Oligopeptide Carriers (SOCs) ".C. Sakarellos. V. Tsikaris. E. Panou-Pomonis. Ch. Alexopoulos. M. Sakarellos-Daitsiotis. C. Petrovas. P.G. Vlachoyiannopoulos and H.M. Moutsopoulos. Letters in Peptide Science. 4, 447-454 (1997)
4. "A Major Sm Epitope Enchored to Sequential Oligopeptide Carriers in a Suitable Antigenic Substrate to Detect Anti-Sm Antibodies".C.J. Petrovas, P.G. Vlachoyiannopoulos. A.G. Tzioufas. Ch. Alexopoulos. V. Tsikaris. M. Sakarellos-Daitsiotis, C. Sakarellos and H.M. Moutsopoulos. J. Immunol. Methods 220, 59-68 (1998).
5. "Peptide Carrier: A New Helicoid-Type Sequential Oligopeptide Carrier (SOCn) for Multiple Anchoring of Antigenic Immunogenic Peptides".M. Sakarellos-Daitsiotis, V. Tsikaris, P.G. Vlachoyiannopoulos. A.G. Tzioufas, H.M. Moutsopoulos, and C. Sakarellos .«Methods»: A Companion to «Methods in Enzymology». Special issue on «Developments in Adjuvant Technology», Topic: «Peptide Carriers». (D. Boraschi and A. Tagliabue, Eds.), Academic Press, 1999. in press.

Tomikazu Sasaki, Ph.D.

Department of Chemistry, Box 351700, University of Washington, Seattle, WA 98195,
Tel: 206-543-6590, FAX: 206-685-8665, E-mail: sasaki@chem.washington.edu
http://www.chem.washington.edu/faculty_pages/sasaki.html

Template approach can bring an orderliness to otherwise flexible and random system. Stabilized peptide a-helices and b-sheets have been constructed on a porphyrin template. Porphyrin ring acts as a structural constraint as well as a spectroscopic probe for the peptide secondary structure. Metal templates have been used to display multiple carbohydrate residues for the binding to lectins and receptors. Labile metal complexes allow the carbohydrate residues to adjust their spatial orientation on the metal template to fit into their binding pocket. The metal templates have also been used for molecular imprinting to construct specific biorecognition sites on a solid surface. Cyclic peptides and porphyrins are a key element for the design of new biomaterials. These template molecules bind to a solid surface to self-assemble to generate a nano-scale pattern. The patterned surface is modified with a biorecognition group to trigger specific biochemical reactions that leads to the healing response.

Tomikazu Sasaki, Ph.D. Dr. Tomikazu Sasaki received his Ph.D. in Synthetic Organic Chemistry in 1985 at Kyoto University with Professor Iwao Tabushi. He was a postdoctral fellow with Professor Emil T. Kaiser at the Rockefeller University from 1985 - 1989. At the Rockefeller, he was involved in the development of a novel segment condensation approach for the chemical synthesis of proteins. He was appointed as an Assistant Professor in Chemistry at the University of Washington in 1989, and promoted to the rank of Associate Professor in 1995. He is an Investigator in the Center for NanoTechnology and in University of Washington Engineered Biomaterials (UWEB). He is a Visiting Professor at Nagoya University in Japan. He is a Scientific Advisor for Syntrix in Seattle. He is members of ACS, AAAS, the Society for Molecular Imprinting and the Forum of Bioinspired Chemistry (Chemical Society of Japan).

Selected Publications:

1. Dynamic Structure and Potential Energy Surface of a Three-Helix Bundle Protein, M. Lieberman, M. Tabet, T. Sasaki, J. Am. Chem. Soc. 116, 5035-5044 (1994).
2. Imprinting for the Assembly of Artificial Receptors on a Solid Surface, Ki-Oh Hwang, T. Sasaki J. Material Chem. 8, 2153 (1998)
3. Template-Assisted Nano-patterning of Solid Surfaces, M. S. Boeckl, T. Baas, K.-O. Hwang, A. L. Bramblett, B. D. Ratner, J. W. Rogers, T. Sasaki Biopolymers (Peptide Science) 47, 185 (1998)
4. G. R. Geier III, T. Sasaki,"Catalytic Oxidation of Alkenes with a Surface-Bound Metalloporphyrin-Peptide Conjugate" Tetrahedron 55, 1859 (1999).
5. S. Sakai, Y. Shigemasa, T. Sasaki "Iron(II)-assisted Assembly of Trivalent GalNAc Clusters and Their Interactions with GalNAc-specific Lectins" Bull. Chem. Soc. Jpn in press (1999).

Thomas Scheibel, Ph.D.

tscheibe@midway.uchicago.edu
HHMI, The University of Chicago
Tel.: 773-702 8795

Structural properties of the NM-domain of yeast Sup35

Thomas Scheibel and Susan Lindquist

The [PSI+] factor of S. cerevisiae represents a new concept of inheritance in which cytosolic transmission of a phenotype is due to an altered protein structure. [PSI+] is regarded as a self-perpetuating conformationally altered form of the chromosomally encoded Sup35 protein, a subunit of the translation-termination apparatus. Sup35 consists of three domains. Two of them, the N-terminal (N) and the middle (M) domain, seem to be critically involved in [PSI+] formation in vivo. However, not much is known about the structural properties of these domains. The N-domain contains several oligopeptide repeats and has a very unusual amino acid distribution. The M-domain is highly charged and secondary structure prediction indicates a large a-helical content in this domain. In vitro aging of recombinant NM expressed in E. coli results in a shift of its CD signal to a form indicative of b-sheet structure. This conformational conversion is accompanied by formation of fibrils. To investigate the structural properties and conformational changes of NM several cysteine mutants of NM (which naturally lacks cysteine) were engineered. These mutants will enable specific fluorescent labeling. The environmental changes of the label will be monitored allowing detailed studies of conformational changes in NM. The cysteine mutants will also be used to immobilize purified NM to directly investigate fiber-formation with various biophysical methods.

Joel Schnur, Ph.D.

Center for Bio/Molecular Science and Engineering, Code 6900
Naval Research Laboratory
Washington, DC 20375, USA
Tel: 202-404-6000,FAX: 202-404-8897,
jschnur@cbmse.nrl.navy.mil http://cbmsews1.nrl.navy.mil

The Center for Bio/Molecular Science and Engineering (1) (Naval Research Laboratory Code 6900) uses the tools of modern biology, physics. chemistry, and engineering to develop advanced materials, sensors, and devices. The long-term research goal is to gain a fundamental understanding of the relationship between molecular architecture and the function of materials. The key theme is the study of complex bio/molecular systems with the aim of understanding how "biology" has approached the solution of difficult structural and sensing problems. Technological areas currently being studied include combat casualty care, environmental quality, biological warfare defense, biofouling retardation, real-time holography, nonlinear-optic materials, spatial-light modulators for mine countermeasures, high-resolution integrated circuit and optoelectronic-device fabrication, high-density microelectronic packaging, filtration systems for oil-water separation, flexible liquid-crystal displays, and large-area, high-information-content flat-panel displays. Center personnel have extensive interactions with other government facilities, as well as industrial and academic laboratories.

Dr. Schnur studied physics and chemistry at Rutgers and Georgetown University receiving his AB in 1966, MS in 1969, and PhD. in 1971. Dr. Schnur then furthered his education with postdoctoral fellowships at the Naval Research Laboratory, Istituto di Fisica, Parma, Italy, (where he played tennis as well) and the Universite' de Paris, Sud until 1973. A National Academy of Science Fellowship awarded to Dr. Schnur supported these studies. Dr. Schnur returned to the United States in 1973 to accept a position in the Optical Sciences Division of the Naval Research Laboratory. Between 1973 and 1983 he pursued research in the area of advanced spectroscopies applied to the study of complex organic materials. In 1983 he was awarded a Professor Associe' at the Universite' of Paris VI. In 1984 he initiated bio/molecular science and engineering research at the NRL. He has been the director of the Center (CBMSE) since its inception.

Dr. Schnur's research interests in the past several years have focussed on molecular self-assembly and the role of molecular shape in the architecture of microstructures. His past work on lipid tubule structure and function has led to some new insights into the role that chirality plays in such structures, as well as posing new questions about the role of chirality and symmetry breaking in molecular microstructures.

Selected Publications:

1. "The Center for Bio/Molecular Science and Engineering," Supramolecular Science **1**: 63-65 (1995).
2. "Controlling the Morphology of Chiral Lipid Tubules", M. Spector, A. Singh, J. Rodriguez, R. Price, and J. Schnur, Langmuir, **14**, 3493-3500 (1998)
3. "DNA Ordering on a Lipid Membrane," M. Spector, J.M. Schnur, Science **275**: 791-792 (1997).
4. "Cooperative Chiral Order in the B-Z Transition in Random Sequences of DNA," J.V. Selinger and J.M. Schnur, Biophysical Journal **73**: 966-971 (1997).
5. "Thermodynamics of Phospholipid Tubules in Alcohol/Water Solutions," MS Spector, J.V. Selinger, J.M. Schnur, Journal of the American Chemical Society **119**: 8533-8539 (1997)
6. "Theory of Cylindrical Tubules and Helical Ribbons of Chiral Lipid Membranes," J.V. Selinger, FC MacKintosh and J.M. Schnur, Physical Review E **53**: 3804-3818 (1996).
7. "Chiral molecular self-assembly of lipid tubules: A circular dichroism study", M. Spector, K. Easwaren, G. Jyothi, J. Selinger, A. Singh, and J. Schnur, PNAS, **93**, 1243-12946 (1996)
1. "Diacetylenic Lipid Tubules: Experimental Evidence for a Chiral Molecular Architecture," J.M. Schnur, BR Ratna, J.V. Selinger, A. Singh, G. Jyothi, and K.R.K. Easwaren, Science **264**:945-947 (1994).
9. "Lipid tubules: A paradigm for Molecularly Engineered Structures" Science, **262**, pp1669- 1676 (1993)

Yechiel Shai, Ph.D.

Department of Biological Chemistry
The Weizmann Institute of Science
Rehovot, 76100 Israel.
Tel: 972-8-9342711; Fax: 972-8-9344112
bmshai@weizmann.weizmann.ac.il

Protein-membrane interactions and peptide-peptide interactions within the lipid environment play a major role in numerous biological events that take place in the cell membrane. (1) Studies with ion channels identified specific molecular recognition within the membrane milieu, between their synthetic segments. This information served to propose structural models for these proteins that agreed well with molecular biology and crystallographic studies. (2) Two distinct mechanisms for membrane permeation by cytolytic polypeptides were identified; (i) channel/pore formation via a "barrel-stave" mechanism by non-cell selective cytolysins which do not discriminate between charged or zwitterionic membranes, and specifically self-associate within the membrane miliue. (ii) membrane disruption via a "carpet" mechanism by bacteria-selective lytic peptides isolated from insects mammals and de-nove designed. (3) Studies with intact and synthetic fragments of envelope proteins of viruses (which promote the fusion of viral and target cell membranes), allowed to propose structural models that describe the different steps involved in the virus-cell fusion process.

Yechiel Shai is an Associate Professor at the Department of Biological Chemistry at the Weizmann Institute of Science. He received his Ph.D. and MSc in Chemistry at the Weizmann Institute and then moved to NIH, Bethesda, as a NIH fellow for three years. He is a member of the Protein Society and the Biophysical Society.

Selected Publications:

1. Shai, Y. "Molecular Recognition Between Membrane - Spaning Polypeptides". TIBS, 20, 460-464(1995).
2. Rapaport, D., 3 Ovadia, M., & Shai, Y. "A Synthetic Peptide Corresponding to a Conserved Heptad Repeat Domain is a Potent Inhibitor of Sendai Virus-Cell Fusion: An Emerging Similarity with Functional Domains of other Viruses" EMBO J. 14, 5524-5531 (1995).
3. Shai, Y., & 1Oren, Z. "Diastereomers of Cytolysins: A Novel Class of Highly Potent Antibacterial Peptides" J. Biol. Chem. (communication) 271, 7305-7308 (1996)
4. Rapaport, D., 1Peled, R., 3Nir, S. & Shai, Y. "Reversible Surface Aggregation in Pore Formation by Pardaxin"Biophys. J. 70, 2502-2512 (1996)
5. Ben-Efraim, I., & Shai, Y. "The Structure and Organization of Synthetic Putative Membranous Segments of ROMK1 Channel in Phospholipid Membranes" Biophys. J. 72, 85--96 (1997)
6. Pritsker, M., Jones, P., Blumenthal, R., and Shai, Y. "A Synthetic All D-Amino Acid Peptide Corresponding the N-Terminal Sequence of HIV-1 gp41 Recognizes the Wild Type Fusion Peptide in the Membrane and Specifically Inhibits HIV-1 Envelope Glycoprotein-Mediated Cell Fusion" Proc. Natl. Acad. Sci. USA, 95, 7287-7292 (1998).
7. Gazit, E., La, R. P., Sansom, M. S. & Shai, Y. The structure and organization within the membrane of the helices composing the pore-forming domain of Bacillus thuringiensis delta- endotoxin are consistent with an "umbrella-like" structure of the pore. Proc. Natl. Acad. Sci. USA, 95, 12289-12294 (1998).
8. Ben Efraim, I., Kliger, Y., Hermesh, C. & Shai, Y. Membrane-induced step in the activation of Sendai virus fusion protein. J. Mol. Biol., 285, 609-625 (1999).

John W Taylor, Ph.D.

Department of Chemistry, Rutgers University
610 Taylor Rd., Piscataway, NJ 08855, USA
Tel: 732-445-0514, FAX: 732-445-5312
taylor@rutchem.rutgers.edu

A major objective of the lab is the development of approaches to understanding the functional conformations of intermediate-sized, flexible peptides with important biological activities. The major approach currently being exploited is the design, synthesis and study of multicyclic peptides as conformationally constrained models of the natural systems. Cyclic structural elements, usually lactam bridges linking pairs of side chains, are incorporated during peptide chain buildup by solid-phase or solution-phase synthetic methods. Recently, for example, we have developed a rigid non-natural linker that stabilizes the _-helical conformation by linking residues separated by two turns of the helix, and we have shown that certain combinations of two overlapping side-chain linkages can essentially lock the peptide backbone in an _-helical conformation. Studies of such side-chain cyclized and other synthetic peptide models have been used to (a) determine accurate thermodynamic parameters for the helix-coil transition, (b) design helix-stabilized and helix-locked analogues of DNA-binding peptides, (c) investigate the active conformations of several peptide hormones, including neuropeptide Y, _-endorphin and calcitonin, and (d) investigate computed models for the folded conformations of G-protein coupled receptors by disulfide trapping.

Dr. Taylor received his Ph.D. in Organic Chemistry in 1983 at the university of Chicago with Professor E. T. Kaiser. He was a Postdoctral Fellow with Professor Michel Lazdunski at the University of Nice, from 1983 to 1984, and then with Professor Fritz Eckstein at the Max-Planck-Inst. for Experimental Medicine, in Gottingen, from 1984 to 1985. He was appointed as an Assistant Professor in the Laboratory of Professor E. T. Kaiser at the Rockefeller University from 1985 until 1991. Since 1991, he has been at Rutgers University at New Brunswick, where he was appointed as an Associate Professor in the Department of Chemistry and the Waksman Institute.

Selected Publications:

1. Ösapay, G. and Taylor, J. W.: Multicyclic polypeptide model compounds. 2. Three Lys^{i}, Asp^{i+4}, but not Lys^{i}, Glu^{i+4}, lactam bridges generate a highly stable helical conformation in a model amphiphilic peptide. *J. Amer. Chem. Soc.*, **114**, 6966-6973, 1992.
2. Bracken, C., Gulyás, J., Taylor, J. W. and Baum, J.: Synthesis and NMR structure determination of an _-helical, bicyclic, lactam-bridged hexapeptide. *J. Amer. Chem. Soc.* **116**, 6431-6432, 1994.
3. Kapurniotu, A. and Taylor, J. W.: Structural and conformational requirements for human calcitonin activity: design, synthesis and study of lactam-bridged analogues. *J. Med. Chem.*. **38**, 836-847 1995.
4. Lu, J. and Taylor, J. W.: Phospholipid vesicle binding by an amphiphilic _-sheet structure from serum apolipoprotein B-100. *Proc. 15th Amer. Peptide Symp.* (J. P. Tam & P. T. P. Kaumaya, eds.), Kluwer Academic Publishers, 1998, pp. 563-564.
5. Yu, C. and Taylor, J. W.: Synthesis and study of peptides with semirigid i and i+7 side-chain bridges designed for _-helix stabilization. *Bioorg. Med. Chem.* **7**, 161-175, 1999.

Bruce Tidor, Ph.D.

Department of Chemistry, Room 6-135
Massachusetts Institute of Technology
Cambridge, MA 02139-4307, USA
Tel: 617-253-7258, FAX: 617-252-1816,
tidor@mit.edu http://mit.edu/tidor/

Dr. Tidor's research focuses on the structure, function, and interactions of proteins and nucleic acids and their complexes. Using molecular modeling theory and computation, he explores the roles played by specific chemical groups in defining the stability and specificity of molecular interactions. He is actively involved in applying knowledge from modeling studies to rational design. Investigations probe the sources of stability and specificity that drive folding and binding events of macromolecules. Studies are aimed at dissecting the interactions responsible for the specific structure of folded proteins and the binding geometry of molecular complexes. The roles played by salt bridges, hydrogen bonds, side-chain packing, rotameric states, solvation, and the hydrophobic effect in native biomolecules are being explored, and strategies for re-casting these roles through structure-based molecular design are being developed. The methods of theoretical and computational biophysical chemistry are the primary tools for research in this laboratory, supplemented by algorithms from artificial intelligence and applied mathematics.

Bruce Tidor graduated summa cum laude in Chemistry and Physics from Harvard College in 1983, and then received a Marshall Scholar award to study at Oxford University's Wolfson College, where he earned an M.Sc. in Biochemistry. He received his Ph.D. in Biophysics from Harvard in 1990 and moved to the Whitehead Institute for Biomedical Research, where he started his independent research as a Whitehead Fellow. In 1994 he was appointed an Assistant Professor in the Department of Chemistry at MIT. He was an awardee in the University Exploratory Research Program sponsored by Procter and Gamble and has been appointed an Alfred P. Sloan Research Fellow.

Selected Publications:

Z.S. Hendsch and B. Tidor. Do Salt Bridges Stabilize Proteins? A Continuum Electrostatic Analysis. *Protein Sci.* **3:** 211-226 (1994).

Z.S. Hendsch, T. Jonsson, R.T. Sauer, and B. Tidor. Protein stabilization by removal of unsatisfied polar groups: Computational approaches and experimental tests. *Biochemistry* **35:** 7621-7625 (1996).

L.-P. Lee and B. Tidor. Optimization of electrostatic binding free energy. *J. Chem. Phys.* **106:** 8681-8690 (1997).

B. Tidor. Molecular dynamics simulations. *Current Biology* **7:** R525-R527 (1997).

L.T. Chong, S.E. Dempster, Z.S. Hendsch, L.-P. Lee, and B. Tidor. Computation of electrostatic complements to proteins: A case of charge stabilized binding. *Protein Sci.* **7:** 206-210 (1998).

C.V. Sindelar, Z.S. Hendsch, and B. Tidor. Effects of salt bridges on protein structure and design. *Protein Sci.* **7:** 1898-1914 (1998).

E. Kangas and B. Tidor. Optimizing electrostatic affinity in ligand–receptor binding: Theory, computation, and ligand properties. *J. Chem. Phys.* **109:** 7522-7545 (1998).

P.B. Harbury, J.J. Plecs, B. Tidor, T. Alber, and P.S. Kim. High-resolution protein design with backbone freedom. *Science (Washington, D.C.)* **282:** 1462-1467 (1998).

E. Kangas and B. Tidor. Charge optimization leads to favorable electrostatic binding free energy. *Phys. Rev. E* **59:** 5958--5961 (1999).

Z. S. Hendsch and B. Tidor. Electrostatic interactions in the GCN4 leucine zipper: Effects of intramolecular interactions that are enhanced on binding. *Protein Sci.*, in press.

Dan W. Urry, Ph.D.

Department of Chemical Engineering and Materials Science (612-625-4540)
151 Amundson Hall, 421 Washington Ave. S.E., Minneapolis, MN 55455-0132
Biological Process Technology Institute (612-625-4282), 240 Gortner Laboratory,
1479 Gortner Avenue, College of Biological Sciences,
St. Paul, MN, 55108
University of Minnesota, Twin Cities Campus
danurry@cems.umn.edu

Our research has experimentally developed five axioms for protein (and amphiphilic polymer) engineering and function, has demonstrated the dominant responsible mechanism to be competition for hydration between hydrophobic and polar groups, has designed elastic molecular machines capable of interconverting the set of the energies interconverted in metabolism, and is employing these capacities to design protein-based polymers for medical and non-medical applications.

Protein-based materials can be designed to exist as hydrogels, elastomers and plastics that can be interconverted. Representative applications under development are: soft tissue restoration, prevention of post-surgical adhesions, drug delivery, thermoplastics that are programmable for biodegradation, and free energy transducers designable to function simultaneously as sensor and actuator involving the intensive variables of mechanical force, temperature, pressure, chemical potential, electrochemical potential, and electromagnetic radiation. Soft tissue restoration utilizes the concept of temporary functional scaffoldings and includes multiple approaches to restoring intervertebral disc function, urinary continence, small caliber vessels, etc.

Dr. Urry received his Ph.D. in Physical Chemistry form University of Utah. He is currently Professor of Chemical Engineering and Member, Biological Process Technology Institute, University of Minnesota, 1997-Present. He was Director of Laboratory of Molecular Biophysics, University of Alabama at Birmingham, School of Medicine, 1970 – 1997, and Professor of Biochemistry, Physiology and Biophysics, University of Alabama at Birmingham, School of Medicine, 1970 – 1997. He has won Wright A. Gardner Award, 1991; Humboldt Preis, 1979. Scientist of the Year, Research & Development Magazine, 1988, University of Alabama at Birmingham Distinguished Faculty Lecturer, 1987, Listed in Current Contents, No. 21, May 24, 1982, pp. 5-13, as one of the 10 most cited scientists in Biophysics and No. 41, October 12, 1981, pp. 5-14, as one of "The 1,000 Contemporary Scientists Most-Cited 1965-1978." He is members of Phi Beta Kappa; Phi Kappa Phi; Phi Eta Sigma; and won University of Utah Sigma Xi Award, 1963.

Selected publications (from over 450)

1. Dan W. Urry, Asima Pattanaik, Jie Xu, T. Cooper Woods, David T. McPherson and Timothy M. Parker, "Elastic Protein-based Polymers in Soft Tissue Augmentation and Generation," *J. Biomater. Sci. Polymer Edn*, 9, 1015-1048 (1998).
2. Dan W. Urry, "Physical Chemistry of Biological Free Energy Transduction as Demonstrated by Elastic Protein-based Polymers," invited FEATURE ARTICLE, *J. Phys. Chem.B*, 101, 11007-11028, 1997.
3. Dan W. Urry, "Five Axioms for the Functional Design of Peptide-Based Polymers as Molecular Machines and Materials: Principle for Macromolecular Assemblies," *Biopolymers (Peptide Science)*, 47, 167-178 (1998).
4. Dan W. Urry, "Elastic Molecular Machines in Metabolism and Soft Tissue Restoration," *TIBTECH (Trends in Biotechnlogy)*, 17, 249-257 (1999).

Horst Vogel, Ph.D.

Institute of Physical Chemistry, Swiss Federal Institute of Technology,
CH-1015 Lausanne, Switzerland
Tel: (+41) 21-6933155; Fax: (+41) 21-6936190;
horst.vogel@epfl.ch

The laboratory concentrates on three major research topics ranging from **(i)** fundamental studies on the **structure and dynamics of membrane proteins,** to **(ii)** the investigation of the **formation of supramolecular structures at interfaces** by selective self-assembly processes and **(iii)** applied research in the field of **biosensor development.**

10. We are developing a new and general method for protein structure determination by fluorescence spectroscopy . A fluorescent, non-natural amino acid is introduced at a known site into the receptor protein by molecular biological methods. Subsequently, intermolecular distances are determined by measuring the fluorescence resonance energy transfer between the fluorescent non-natural amino acid in the receptor and a suitably fluorescently labeled ligand molecule which bind to the receptor. This allows the determination of distances and thus defines the three-dimensional structure of the receptor, either in reconstituted systems or even intact living biological cells. (Turcatti et al., *J. Biol. Chem. 271*[1996] 19991-19998; Tairi et al., *Biochemistry 37* [1998] 15850-15864)

(ii) Two-dimensional micro-scale structures of receptors on solid supports are formed using one or several of the following methods: Langmuir-Blodgett technique, photo- or electron beam lithography, micro-contact printing and the formation of self-assembled (supra-) molecular layers taking advantage of highly specific molecular interactions. Our motivation in creating these surface structures is firstly to understand complex self-organization processes of biopolymers at interfaces, and secondly to create micro- and nano-sized multichannel arrays which find applications in fields such as biosensors and novel (biocompatible) materials. (Heyse et al., *Biochemistry 37*[1998] 507-522; Liley et al., *Science 280* [1998] 273-275)

(iii) Most of the existing biosensing principles rely on enzymatic reactions or antigen-antibody interactions in order to detect the analytes of interest. In order to overcome the limitations of traditional biosensor applications, novel concepts have to be found to improve the biosensors' stability, to increase their sensitivity by applying novel physical detection techniques and most importantly, to open biosensors to general biological signal recognition, transduction, and amplification principles. Our research has entered a novel field of biosensors by applying biological signal recognition and amplification processes which are based on either natural membrane protein receptors (ion channel proteins, G protein-coupled receptors). Our recent activities concentrate on the detection of single receptor molecule detection by fluorescence spectroscopy. (Schmid et al., *Anal. Chem. 70* [1998] 1331-1338; Heyse et al., *Biochim. Biophys. Acta 1376* [1998] 319-338 ; Kröger et al., *Anal. Chem.* in press; Wohland et al., *Biochemistry*, in press).

Horst Vogel studied Chemistry at the University of Würzburg/Germany. After his diploma thesis in Physical Chemistry he went to the Max-Planck-Institute for Biophysical Chemistry in Göttingen where he performed his PhD work on the "Structure of lipid membranes" in the Departments of Profs. M. Eigen and A. Weller. In the following he worked at the Max-Planck-Institute for Biology in Tübingen, Germany, at the Biocenter of the University of Basel, Switzerland, and at the Karolinska Institute in Stockholm, Sweden, studying the structure and dynamics of membrane proteins. In 1994 he was appointed as a Professor of Physical Chemistry at the Swiss Federal Institute of Technology (Ecole Polytechnique Fédérale) in Lausanne, Switzerland, where he is director of the Institute for Physical Chemistry of Polymers and Membranes.

Keith B. Ward, Ph.D.

Biomolecular and Biosystems S&T Division
Office of Naval Research-335
800 N. Quincy Street
Arlington, VA 22217-5660
(703) 696-0361; Fax: (703) 696-1212
Keith_Ward@onr.navy.mil

The Office of Naval Research has a proud history of supporting groundbreaking fundamental research in a wide variety of areas. Recent changes in organization have led to increasing interest in developing future Naval technology options by supporting applied research efforts as well. A major emphasis is placed upon building interdisciplinary teams of principal investigators working to address different aspects of a common technology problem in strongly-coupled, cooperative projects. A current topic of interest seeks to understand what unique functions and capabilities biological materials and processes can bring to both meso- and nanoscaled devices. How can biology best be incorporated into silicon-based architectures? Almost certainly self-assembly of proteins will play a major role in achieving an initial goal of designing and constructing prototype, proof of concept, devices that demonstrate effective integration of biological materials or biological processes, at the nanoscale, into Sili-Bio, hybrid systems.

Keith B. Ward was trained as a Biophysicist in Warner Love's protein crystallography laboratory at Johns Hopkins University. He led the Macromolecular Structure and Function group at the Naval Research Laboratory until joining ONR in 1996. He manages Basic Research programs in Novel Biomolecular Materials and serves as the Program Manager of ONR's Biosensors and Biomaterials Applied Research Program, as well as directing the Chemical Sensing in the Marine Environment technology development project.

Abraham Warshawsky, Ph.D.

cowarsha@wiccmail.weizmann.ac.il
Department of Organic Chemistry
Weizmann Institute of Science
Rehovot, Israel 76100

Dr. Warshawsky received his education at the, Faculty of Chemistry, Israel Institute of Technology, Haifa,Israel, 1960-1969: B.Sc. M.Sc D.Sc After a period of three years(1970-1973) as Scientist,and Principal Scientist, National Institute for metallurgy (N.I.M), Johannesburg, South Africahe joinded the Department of Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel (W.I.S.)he holds ,the Rebbeca and Israel Sieff Professorial Chair in Organic Chemistry. In 1980/81: 86/7 he visited the Chemistry Division and Life Sciences Division Eastman-Kodak Research Laboratories, Rochester, New York, U.S.A, Rochester 1991-1999 coEditor-in-Chief – Reactive Polymers and Reactive@Functional Polymers .he has many1990's Visiting professorship including: Imperialcollegue, Cambridge, Northwestern University, University Autonoma, Barcelona. Society of Chemical Industry (London), and corresponding member,Israel and Middle East, for the ion exchange and solvent extraction group of the society. American Chemical Society, subdivisions of Separation & Science Guest Editor , Israel J. Chemistry, "Specific Ion ExchangMaterials and Systems".(1985)Guest co-editor Reactive polymers, ion exchangers, sorbents. Special issue Vol. 6, Nos. 2 and 3, 1987.Guest co-editor Drug delivery and slow release (special issue) Reactive Polymers, Elsevier, Vol. 25 (,1994) Co-editor 5th Spanish-Italian and Mediterranean Basin Congress (SIMEC '94). React. Funct. Polym. 1998Guest co-editor, Ion Exchange Theory and Practice, Advances of Russian Scientists, in: Solvent Ion Extraction, Dekker, New York, Vol. 16.Co-editor (together with S.D. Alexandratos) State-of-the-Art: Selective Ion-exchangers, React. Funct. Polym., Vol.36 (2)(1998)Co-editor Hogfeldt Memorial Issue, React. Funct. Polym..(1998)Co-editor Special Issue dedicated to "Polymer Science in Israel", Actapolymerica, October 1998.Co-editor High lights in Russian Science. Vol. I. Ion Exchange, Dekker, New York, due for publication in 1999 guest coeditor Vol. II, Isotope Separations, Dekker, New York, due for publication in 1999.Guest coeditor with M.Fridkin,POC98 special issue R@FP,1999.Co-chairman ,IUPAC Conference on "Organic Chemistry of Technological Processes", Jerusalem, June 1-6, 1986.Co-chairman ,Third International Conference on "Polymer Supported Reactions in Organic Chemistry", Jerusalem, July 6-11, 1986.Co-chairman ,1st Russian-Israel Symposium on Polymer Science and Technology, Rehovot.(1993)Co-chairman ,23rd Aharon Katzir-Katchalsky Conference. International Conference on Environmental Impact of Polymeric Materials. Rehovot, May 12-16, 1996 Co-chairman)8th International Conference on Polymer Based Technologies (POC '98), and the 26th Annual Katzir-Katchalsky Conference, Ma'ale Hachamisha, Israel, June 28 - July 3,Five relevant publications: N. Kahana, R. Arad-Yellin and A. Warshawsky. A conceptual approach to the synthesis of bifunctional EDTA analogs: EDTA-extended polyamides. J. Org. Chem. 59, 4832-4837 (1994).I. Rogachev, V. Kampel, V.. Gusis, N. Cohen, J. Gressel and A. Warshawsky Synthesis, properties and use of copper-chelating amphiphilic dithiocarbamates as synergists of oxidant-generating herbicides, Pesticide Biochem. and Physiol. 60,135-146 (1998). Rogachev, L. Weiner, J. Gressel and A. Warshawsky.inhibition of Cu/Zn superoxide dismutase activities by amphiphilic dithiocarbamates Archives of Biochemistry and Biophysics (1999) submitted S. Litvin, L. Weiner and A. Warshawsky An EDTA derivative of phosphotitdylethanolamine - a new metal chelating agentJ. Chem. Soc., Perkins Trans.to be submitted(1999).L. Bromberg, I. Lewin, H. Gottlieb and A. Warshawsky Interaction of mercury (II) and silver (I) with bis[di(2-ethylhexyloxy)-thiophosphoryl]disulfide.Inorganica Chimica Acta, 197, 95-99 (1992) L. Bromberg, D. Muraviev and A. WarshawskMembrane-assisted D-H exchange reaction on trimethylamineborane. J. Phys. Chem. 97, 967-971 (1993).

David S. Wilson, Ph.D.

Department of Genetics
Harvard Medical School/ MGH
Boston, MA USA
wilson@genetics.mgh.harvard.edu

"mRNA-display" is a method developed in Jack Szostak's research group in which up to 10^{14} different protein molecules can be individually tagged by their encoding mRNAs (1). This linkage is accomplished by chemically attaching puromycin to the 3' end of the mRNAs in a library. When these RNAs are translated in vitro, the nascent proteins become covalently linked to their encoding mRNAs via the puromycin moiety. These chimeric molecules can then be partitioned by employing selection schemes for binding or catalytic properties.

The Szostak laboratory uses this technique to probe the density and distribution of functional sequences within polypeptide sequence space. In particular, we are interested in the following questions: what fraction of proteins from a random sequence library can accomplish a given function (binding or catalysis)? Can libraries be designed so as to increase the frequency of functional molecules? How many and what kinds of mutations are required to improve or change a protein's biochemical activity?

David Wilson is a post-doctoral fellow in the Szostak lab, and is currently working with libraries of proteins composed of short amphipathic alpha-helix and beta-strand-forming sequences. Prior to this, he did his doctoral work at The Rockefeller University (1989-1995). Here he focused on protein-DNA interactions, combining biochemical analysis (Laboratory of Claude Desplan; 2-3) with the 3-D structure determination of a protein-DNA complex using X-ray crystallography (Laboratory of John Kuriyan; 4).

(1) Roberts, R.W. & Szostak, J.W. *PNAS* 94:12297 (1997).
(2) Wilson, D., Sheng, G., Lecuit, T., Dostatni, N. & Desplan, C. *Genes & Devel* 7:2120 (1993).
(3) Wilson, D.S., Sheng, G., Jun, S. & Desplan, C. *PNAS* 93:6886 (1996).
(4) Wilson, D.S, Guenther, B., Desplan, C. and Kuriyan, J. *Cell*, 82: 709 (1995).

Shuguang Zhang, Ph.D.

Center for Biomedical Engineering 56-341
Massachusetts Institute of Technology
77 Massachusetts Avenue
Cambridge, Massachusetts 02139-4307
Tel: 617-258-7514, FAX: 617-258-0204
shuguang@MIT.EDU, http://web.mit.edu/shuguang/www

Biological self-assembly systems lie at the interface between molecular biology, chemistry, materials science and engineering. The key elements in molecular self-assembly are chemical complementarity and structural compatibility. Several distinctive types of peptides have been developed. Type I peptides undergo intermolecular self-assembly to form a gel matrix that can be used for as a scaffold tissue engineering. Type II peptides undergo structural transformations for intermolecular and intramolecular self-assembly. Analysis of the molecular switch will provide us insight into the onset of protein conformational diseases. Type III peptides undergo self-assembly on to surfaces to form molecular hook and molecular Volcro that interact with other molecules and to control cell patterns. Type IV peptides undergo assembly with nucleic acids to promote gene delivery. The self-assembling peptide systems are simple, versatile and easy to produce. These systems represent a significant advancement in the molecular engineering of protein fragments for diverse technological innovations.

Shuguang Zhang is the Associate Director of the Center for Biomedical Engineering at Massachusetts Institute of Technology. He received his Ph.D. in biochemistry and molecular biology from *University of California at Santa Barbara.* He received his B.S. from Sichuan University in China. He was a past American Cancer Society Fellow at MIT. He is a Visiting Professor at Tsinghua (Qinghua) University in Beijing and at Sichuan University in Chengdu, China. He is a consultant for Acorda Therapeutics in New York; and a Scientific Advisor for Mitsubishi Chemical Corporation Research Center in Yokohama, Japan. He is members of AAAS, American Society of Biochemistry and molecular Biology (ASBMB), The Human Genome Organization Americas (HUGO), the Protein Society, New York Academy of Sciences, The International Society for the Study of Origin of Life (ISSOI) and the honorary society of Sigma Xi.

Selected Publications:

Zhang, S., Holmes, T., Lockshin, C. & Rich, A (1993), Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. *Proc. Natl. Acad. Sci. USA* **90**, 3334-3338.

Zhang, S. & Egli, M. (1995) A proposed complementary pairing mode between single-stranded nucleic acids and beta-stranded peptides: A possible pathway for generating complex biological molecules. *Complexity* **1**, 49-56.

Zhang, S., Holmes, T., DiPersio, M., Hynes, R. O., Su, X., & Rich, A (1995) Self-complementary oligopeptide matrices support mammalian cell attachment. *Biomaterials* **16**, 1385-1393.

Zhang, S & Rich, A. (1997) Direct conversion of an oligopeptide from a beta-sheet to an alpha-helix: A Model for amyloid formation. *Proc. Natl. Acad. Sci. USA* **94**, 23-28.

Zhang, S. Yan, L. Altman, M., Lässle, M., Nugent, H., Frankel, F., Lauffenburger, D., Whitesides, G. & Rich, A. (1999) Biological surface engineering: A simple system for cell pattern formation. *Biomaterials* **20**, 1213-1220.

ACKNOWLEDGEMENT

We gratefully acknowledge the generous support of:

Aviv Foundation

Ellison Medical Foundation

European Peptide Society, UK

Defense Advanced Research Project Agency (DARPA)

European Research Office (ERO)

National Institute of Health/National Institute of Dental Research

National Institute of Health/National Institute of Neurological Disorder & Stroke

National Science Foundation/ Molecular Biochemistry Program

Office of Naval Research (ONR)

The US Army Research Office (ARO)

Bristol-Myers Squibb, Princeton, NJ

Corning, Inc. Science Product Division, Acton, MA

Fujisawa Pharmaceutical, Co. Osaka, Japan

Merck Research Labs, Rahway, NJ

Mologen, Holing AG, Berlin, Germany

New England Biolabs, Beverly, MA